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**VERIFICATION OF A TRANSLATION**

I, Michel PERNELLE, hereby declare that:

I am knowledgeable in the French language in which the below identified international application was filed, and that, to the best of my knowledge and belief, the English translation of the French patent application N° FR99 07935 is a true and complete translation of the above identified international application as filed.

I hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent application issued thereon.

Date: April 13, 2005

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ICBP90 POLYPEPTIDE AND ITS FRAGMENTS AND  
POLYNUCLEOTIDES CODING FOR SAID POLYPEPTIDES AND  
APPLICATIONS TO THE DIAGNOSIS AND TREATMENT OF CANCER

The present invention relates to a new ICBP90 polypeptide and its fragments, to the cloning of cDNA and polynucleotides coding for said polypeptides, to cloning and/or expression vectors including said 5 polynucleotides, cells transformed by said vectors and specific antibodies directed against said polypeptides. The invention also relates to methods and kits for diagnosing cancers, to a method and kit for screening ligands of the polypeptides of the invention and of 10 compounds which may be used as a drug for prevention and/or treatment of cancers.

DNA topoisomerases are highly preserved nuclear proteins during evolution, the main role of which is for controlling DNA conformation and topology in the 15 nucleus, which are constantly altered by the various biological processes involving DNA such as for example, transcription and replication. Topoisomerases exert their action by cutting DNA and linking these lesions after having achieved the adequate conformational 20 change.

In mammals and humans in particular, today, there are at least five different genes coding for a topoisomerase and at least two additional pseudogenes (for a review, see Nitiss 1998). Thus, topoisomerase I, 25 coded by the TOP1 gene removes the superturns present in DNA while only cutting a single strand. Both topoisomerases of type II existing in humans called TopII $\alpha$  and TopII $\beta$ , alter DNA topology by introducing transient double strand cleavages (for a review, see

Wang 1996). Finally, there are two topoisomerases of type III coded by two localized genes in 17p11.2-12 and 22q11-12 and they only act against negative superturns of DNA.

5 In tumoral cells, topoisomerases of type II play a very important role; in these growing and rapidly dividing cells, there is a large need for maintaining DNA molecules in a proper conformation as high transcription and replication rates are required. Thus,  
10 10 the rates for topoisomerase II are generally higher in human tumoral cells than in normal tissues of the same origin. However, the high expression rate of topoisomerase II $\alpha$  in tumoral cells may vary among two tumors of different natures affecting a same tissue.  
15 For example, the nucleus of cells from small cell carcinomas of the lung has a higher rate of topoisomerase II $\alpha$  than the nucleus of cells from lung carcinomas with normal sized cells (Guinee et al., 1996). In the same way, the rate of topoisomerase II $\alpha$   
20 20 in A59 cells is three times higher than in PC3 cells, both of these cell lines stemming from the adenocarcinoma of lung epithelium (Yamasaki et al., 1996).

These observations suggest that topoisomerase II $\alpha$   
25 25 may be considered as a marker of cell proliferation for certain types of cancer. As the cancerous process is characterized by abnormal cell proliferation partly due to the loss of contact inhibition, topoisomerase II $\alpha$  therefore appears as a preferential target for  
30 30 chemiotherapeutical drugs for treating cancer (Pommier et al., 1994), and the present anticancer treatments largely resort to inhibitors of topoisomerases.

Most of these inhibitors exert their cytotoxic effects by stabilizing the DNA cleavage complex. Drugs like anthracyclines [doxorubicin (adriamycin) or epipodophyllotoxins (such as etoposide (VP-16) or 5 teniposide (VM26))], acridines (such as mAMSA) and anthracendiones (for example, mitoxantrone) are examples of drugs which inhibit topoisomerases II which stabilize the cleavage complex. More recently, a new class of inhibitors of topoisomerases II has been 10 developed; these inhibitors act at the level of catalytic activity and no longer by stabilizing the cleavage complex. The drug, fostriecin is an example of one of them (Boritzki *et al.*, 1988). Today these different drugs are used in palliative and curative 15 anticancer treatments.

Nevertheless, one of the major problems encountered in the present anticancer treatments using inhibitors of topoisomerases is the emergence of a resistance to drugs (Kubo *et al.*, 1995). These 20 resistances are either the occurrence of an overexpression of pumps providing efflux of drugs outside the cells before they reach their target (for example; P-glycoprotein, a protein associated with multi-drug resistance (MRP)), or the occurrence of a 25 change in the expression rate of topoisomerase II $\alpha$  (Deffie *et al.*, 1989; Fry *et al.*, 1991), or either both occurrences (for a review, see Isaacs *et al.*, 1998).

One of the aspects of the present invention is therefore to understand the regulatory mechanisms of 30 the expression of the gene of topoisomerase II $\alpha$ , in order to develop an alternative to the phenomenon of resistance to drugs, observed for certain cancers and this with the aim of enhancing the curative and

preventive treatment of cancers.

There are two types of type II topoisomerase which differ in their expression profile; topoisomerase II $\alpha$  (Top II $\alpha$ ) (170 kD), essentially located in the nucleoplasm at the centromer of the mitotic chromosomes, participates in the fundamental biological processes which are replication, condensation of chromosomes and transcription. It seems that topoisomerase II $\beta$  (Top II (180 kD) is rather involved in the transcription of ribosomal RNA, given the nucleolar localization of this enzyme. Both human type II topoisomerases are localized on two different chromosomes (17q21-22 for topoisomerase II $\alpha$  and 3p24 for topoisomerase II $\beta$ ) (Tsai-Plugfelder et al., 1988; Drake et al., 1989; Chung et al., 1989; Jenkins et al., 1992; Austin et al., 1993).

Unlike topoisomerase II $\beta$ , the expression of which is characterized by a relative consistency, topoisomerase II $\alpha$  has a variation of expression depending on the proliferation state of cells and on their position in the cell cycle. Expression of messenger RNA (RNAm) is higher in proliferating cells than in arrested cells in confluence. The expression of topoisomerase II $\alpha$  increases during the S phase of the cell cycle, reaching a maximum at the end of phase G2/M (Goswami et al., 1996), the level of messenger RNA being ten times higher at the end of phase S than during phase G1. Also, there seems to be a coupling between the synthesis and degradation of topoisomerase II $\alpha$  and chromosomal condensation/decondensation (Heck et al., 1988).

Present knowledge concerning control of the gene

of topoisomerase II $\alpha$ , all in all, remains rather scanty. Recently, a promoter region of about 650 base pairs has been described by Hockhauser et al. (1992), it has all the characteristics of a domestic gene, an  
5 absence of TATA box and a moderate content of GC sites (notably the presence of a Sp1 box which may replace the TATA box) are two examples of this. The presence of 5 inverted CCAAT boxes or ICBs is another feature of this type of promoter.

10 Transcription factors interacting with the promoter of the gene of human topoisomerase II $\alpha$  have been described; c-myb (Brandt et al., 1997), p53 (Sandri et al., 1996), ATF (Lim et al., 1998), Sp1 and Sp3 (Kubo et al., 1995) may be mentioned. Whatever the  
15 case, apart from NF-Y (also called CBF, ACF and CP1, references in Isaacs et al., 1996), the transcription factors which act on the ICB sequences of the promoter for the gene of human topoisomerase II $\alpha$  have not yet been identified and characterized; Herzog and Zwellling  
20 (1997) have however revealed two proteins with an apparent molecular weight of 90 kD and 140 kD which bind ICB1 to ICB4 and ICB5, respectively. Isaacs and his collaborators (1996) have suggested that NFY as well as another unidentified protein recognize an ICB  
25 box of the promoter region of the gene of topoisomerase II $\alpha$ ; they have also shown that ICB2 mutations completely suppressed the reduction in promoter activity normally observed in cells arrested in confluence (Isaac et al., 1996). They identified NFY as  
30 a component of a complex induced by the proliferation and which binds *in vitro* to the ICN2 sequence of the promoter of the gene of human topoisomerase II $\alpha$ ,

although NF-Y is always detectable in cells arrested in confluence (Isaacs et al., 1996). They suggested that ICB2 acts as a negative regulator of the promoter of the gene of topoisomerase II $\alpha$  of cells arrested in 5 confluence and that this repression may be suppressed in proliferative cells. The ICB2 box of the promoter of the gene of topoisomerase II $\alpha$  therefore plays a primordial role in the arrest of the normal proliferative process when the cells reach confluence.

10 Transcription factors binding to the ICB sequence as well as the ICB sequence itself therefore form molecular targets for controlling the expression rate of topoisomerase II $\alpha$ . By intervening on these factors, controlling the expression of the gene of topoisomerase 15 II $\alpha$  and cell proliferation consequently may be contemplated.

The object of the present invention is to detect new transcription factors binding to the ICB box involved in the control of cell proliferation.

20 A recent technique called a "simple hybrid" system has been used, which allows DNAc clones coding for the proteins binding to this specific DNA of certain sequences to be isolated. This system has a double advantage as it is able not only to reveal DNA-protein 25 interaction *in vivo* in yeast, but also to give direct access to complementary DNAs (cDNA) coding for the candidate proteins having a transcription factor activity. This system is mainly based on the construct of a test yeast strain according to the principle 30 developed by Wang and Reed (1993). This yeast strain enables DNAc banks to be screened by demonstrating DNA-protein interaction *in vivo* through activation of a

reporter gene integrated within the genome of the test yeast.

The object of the present invention is therefore an isolated polypeptide designated as ICBP90 (inverted CCAAT box binding protein) with the amino acid sequence SEQ ID No.2. This sequence comprises:

a) a "ubiquitin" domain comprising the sequence of amino acids 1-75 of sequence SEQ ID No.2;

b) a "zinc finger" domain of the C4HC3 type comprising the sequence of amino acids 310-366 of sequence SEQ ID No. 2 and a "zinc finger" domain of the C3HC4 type comprising the sequence of amino acids 724-763 of sequence ID No.2;

c) a presumed "zipper leucine" domain comprising the sequence of amino acids 58-80 of sequence SEQ ID No.2;

d) two potential nuclear localization domains comprising the sequences of amino acids 581-600 and 648-670 of sequence SEQ ID No.2;

e) a site for phosphorylation with a tyrosine kinase comprising the sequence of amino acids 452-458 of sequence SEQ ID No.2;

f) sites for phosphorylation with a dependent cAMP/cGMP protein kinase comprising the sequences of amino acids 246-249, 295-298 and 648-651 of sequence SEQ ID No.2;

g) sites for phosphorylation with a casein kinase II comprising the sequence of amino acids 23-36, 57-60, 91-94, 109-112, 165-168, 265-268, 354-357 and 669-672 of sequence SEQ ID No.2;

h) sites for phosphorylation with a protein kinase C comprising the sequence of amino acids 82-84, 104-106, 160-162, 173-175, 251-253, 301-303, 380-382,

393-395, 504-506, 529-531, 625-627 and 639-641 of sequence SEQ ID No.2.

The present invention also relates to an isolated polypeptide characterized in that, it comprises a 5 polypeptide selected from:

- a) a polypeptide of sequence SEQ ID No.2, SEQ ID No.4, SEQ No.6 or SEQ ID No.8;
- b) a polypeptide, a polypeptide variant of sequences of amino acids defined under a);
- 10 c) a polypeptide homologous to the polypeptide defined under a) or b) and including at least 80% homology, preferably 90% with said polypeptide of a);
- d) a fragment of at least 5 consecutive amino acids of a polypeptide defined under a), b) or c);
- 15 e) a biologically active fragment of a polypeptide defined under a), b) or c).

It should be understood that the invention relates to polypeptides obtained through purification from natural sources or else obtained through genetic 20 recombination or even by chemical synthesis and they may then include non natural amino acids.

In the present specification, the term "polypeptide" will be used for also designating a protein or a peptide.

25 The term "polypeptide variant" shall be understood as designating all the mutated polypeptides which may exist in nature, in particular in the human being, and which notably correspond to truncations, substitutions, deletions and/or additions of amino acid residues. The 30 homologous polypeptides according to the invention at least retain a domain selected from the DNA binding domain and/or the interaction domain with another protein.

It shall be understood that the term "homologous polypeptide" designates polypeptides having certain modifications, as compared with the natural polypeptide ECBP90, as in particular a deletion, addition or 5 substitution of at least one amino acid, a truncation, an extension and/or a chimeric fusion. Among the homologous polypeptides, those for which the sequence of amino acids have at least 80% homology, preferably 90%, more preferably 95%, and most preferably 97% 10 homology with the sequences of amino acids of the polypeptides according to the invention, are preferred. In the case of a substitution, one or several consecutive or non consecutive amino acids are replaced with "equivalent" amino acids. Here, the expression 15 "equivalent" amino acid aims at designating any amino acid capable of being substituted for one of the amino acids of the basic structure without however changing the essential functional properties or characteristics, such as their biological activities, of the 20 corresponding polypeptides such that induction *in vivo* of antibodies capable of recognizing the polypeptide for which the amino acid sequence is comprised within the amino acid sequence SEQ ID No.2, or in one of its fragments as defined above, and notably the sequence of 25 amino acids SEQ ID No.4, SEQ ID No.6 and SEQ ID No.8. These equivalent amino acids may be determined either by relying on their structural homology with the amino acids which they replace, or on the results of cross biological activity tests which may take place for the 30 different polypeptides. As an example, the possibilities of substitutions which may carried out without their resulting a deep change in the biological activities of the corresponding modified polypeptides

will be mentioned, for example replacements of leucine with valine or isoleucine, of aspartic acid with glutamic acid, of glutamine with asparagine, of arginine with lysine etc., the reverse substitutions  
5 may naturally be contemplated under the same conditions.

It shall be understood that the term "biologically active fragment" designates in particular a fragment of an amino acid sequence of a polypeptide according to  
10 the invention having at least one of the functional characteristics or properties of the polypeptides according to the invention, notably in that: (i) it is capable of being recognized by a specific antibody of a polypeptide according to the invention; (ii) it has at  
15 least one of the domains or regions as defined above; (iii) it is capable of binding to DNA and notably to the CCAATT and/or inverted CCAAT boxes; (iv) it is capable of modulating the expression rate of the gene of topoisomerase II $\alpha$ , (v) it is capable of modulating  
20 cell proliferation.

It is understood that the term "polypeptide fragment" designates a polypeptide including a minimum of 5 amino acids, preferably 7 amino acids, more preferably 10, and most preferably 15 amino acids.  
25 Fragments of a polypeptide according to the invention, obtained by cleaving said polypeptide with a proteolytic enzyme, with a chemical reagent, or even by placing said polypeptide in a very acid environment, are also part of the invention.

30 The polypeptide according to the invention may also be associated with other polypeptides through protein-protein interactions. It is understood that the term "protein-protein interactions" designate

associations which directly bring into contact at least two proteins. Thus, the polypeptide of the invention may dimerize in order to form homodimers or heterodimers, or be associated as homomultimers or 5 heteromultimers. The polypeptide according to the invention may also interact with another polypeptide in order to exert its action; hence, the polypeptide according to the invention may also have, in addition to its DNA binding domain, a domain acting on the 10 transcription which exerts its action via protein-protein interactions with other protein components of the transcriptional machinery. It is understood that the term "protein component of the transcriptional machinery" designates all transcription factors 15 required for performing and controlling the transcription reaction.

The polypeptide according to the invention is characterized in that it is capable of binding to a DNA sequence and in that it includes at least a DNA binding 20 domain selected from the group consisting of a "zinc-finger" domain and a "leucine zipper" domain; the DNA sequence to which binds said polypeptide is a CCAAT box, preferably an inverted CCAAT box: ICB.

It is understood that the term "binding to a DNA sequence", designates a specific interaction between the polypeptide of the invention and a DNA sequence by means of a series of weak bonds formed between the amino acids of the protein and the bases. The polypeptide according to the invention, has at least a 25 DNA binding domain which contains at least one of the known protein units capable of interacting with DNA, i.e. the zinc-finger structure with which is associated 30 a zinc atom (zinc-finger) the helix-turn-helix

structure, the helix-loop-helix structure, and the leucine-zipper structure.

It is understood that the term "zinc-finger unit" designates a sequence of about twenty amino acids 5 assuming a zinc-finger shape in space. There are two types of them: those which contain four cysteines (C4) and those which contain two cysteines and two histidines (C2H2). These amino acids define the nature of the zinc-finger and they are located at its base and 10 a Zn<sup>++</sup> ion is located in the middle of the square formed by these four amino acids. The polypeptide according to the invention potentially has two units of type C4.

It is understood that the term "leucine zipper 15 type units" designates units belonging to dimeric transcription factors which are either homodimers or heterodimers. The monomer consists of a sequence with a basic character which interacts with DNA in a specific way and of a  $\alpha$  helix hydrophobic domain which interacts 20 with the homologous domain of the other chain. In this domain, leucine is found every 7 amino acids, i.e. at each turn of the helix. All these leucines are aligned and the interaction occurs at their level between both monomers. The polypeptide according to the invention 25 potentially has a leucine zipper type unit.

The invention also relates to an isolated polynucleotide characterized in that it codes for a polypeptide of sequence SEQ ID No.1 as defined earlier. Preferably, the polynucleotide according to the 30 invention has the SEQ ID No.1 sequence.

The invention also relates to the isolated polynucleotide characterized in that it comprises a polynucleotide selected from:

a) a polynucleotide with sequence SEQ ID No.1, SEQ ID No.3, SEQ ID No.5 or SEQ ID No.7 or for which the sequence is that of the RNA corresponding to sequence SEQ ID No1, SEQ ID No.3, SEQ No.5 or SEQ ID No.7;

5 b) a polynucleotide for which the sequence is complementary to the sequence of a polynucleotide defined under a),

10 c) a polynucleotide for which the sequence includes at least 80% homology with a polynucleotide defined under a) or b),

d) a polynucleotide which hybridizes under high stringency conditions with a polynucleotide sequence defined under a), b) or c),

15 e) a fragment of at least 15 consecutive nucleotides, preferably 21 consecutive nucleotides, and more preferably 30 consecutive nucleotides of a polynucleotide defined under a), b), c) or d), except for sequences SEQ ID No.9, No.10 and No.11 corresponding to the human ESTs No. AI 0830773, No. AA 20 811055, No. AA 488 755, No. AA 129 794 and No. AA 354 253 present in the human EST data bases (human dbest), respectively.

In the present specification, it is understood that the terms, "polynucleotide, oligonucleotide, 25 polynucleotide sequence, nucleotidic sequence, or nucleic acid", shall designate a DNA fragment, as well as a double strand DNA, a single strand DNA, as well as transcription products of said DNAs, and/or an RNA fragment, said isolated natural or synthetic fragments 30 whether including non-natural nucleotides or not, designating a specific chaining of nucleotides, whether modified or not, providing definition of a fragment or a region of a nucleic acid.

It is understood that the term "polynucleotide" with a complementary sequence, designates any DNA for which the nucleotides are complementary to those of SEQ ID No.1, SEQ ID No.3, SEQ ID No.5, SEQ ID No.7 or of a part of SEQ ID No.1, SEQ No.3, SEQ ID No.5, SEQ ID No.7 and for which the orientation is inverted.

In the sense of the present invention, it is understood that the term "homology percent" designates a percentage of identity between bases of two polynucleotides, this percentage being purely statistical and the differences between both polynucleotides are randomly distributed throughout their length. According to the invention, the polynucleotides with a homologous nucleic sequence have a homology rate of at least 80%, preferably 90%, more preferably 95%, most preferably 97%.

Hybridization under strong stringency conditions means that the temperature and ionic force conditions are selected in such a way that hybridization between two complementary DNA fragments may be maintained. As an illustration, strong stringency conditions of the hybridization step for the purpose of defining the polynucleotidic fragments described above, advantageously are the following:

DNA-DNA or DNA-RNA hybridization is achieved in two steps: (1) prehybridization at 42°C for 3 hours in phosphate buffer (20 mM pH 7.5) containing 5 x SSC (1 x SSC corresponds to a 0.15 M NaCl + 0.015 M sodium citrate solution), 50% formamide, 7% sodium dodecylsulfate (SDS), 10 x Denhard's, 5% dextran sulfate and 1% salmon sperm DNA; (2) the actual hybridization for 20 hours at a temperature depending on the size of the probe (i.e. 42°C, for a probe with a

size > 100 nucleotides), followed by two washings for 20 minutes at 20°C into 2 x SSC + 2% SDS, one washing for 20 minutes at 20°C into 0.1 x SSC + 0.1% SDS. The last washing is performed in 0.1 x SSC + 0.1% SDS for 5 30 minutes at 60°C for a probe with a size > 100 nucleotides. The strong stringency hybridization conditions described above, for a polynucleotide with a defined size, will be adapted by one skilled in the art for oligonucleotides with a larger or smaller size, 10 according to the teaching of Sambrook et al., 1989.

Advantageously, a nucleotidic fragment meeting the earlier definition will have at least 15 consecutive nucleotides, preferably at least 21 nucleotides, and even more preferably at least 30 consecutive 15 nucleotides of the sequence from which it stems.

It is understood that the term EST ("expressed sequence tag") designates expressed sequences, characterized in a complementary DNA bank (DNAc) and used as a map marker for genomic DNA.

20 According to one embodiment of the invention, the polynucleotide according to the invention is characterized in that it is directly or indirectly labeled with a radioactive compound or a non-radioactive compound. Use of a polynucleotide according 25 to the invention as a primer for amplifying or polymerizing nucleic sequences; the invention also relates to the use of a polynucleotide according to the invention as a probe for detecting nucleic sequences. According to the invention, the polynucleotide 30 fragments may be used as a probe or as a primer in methods for detecting, identifying, dosing and amplifying nucleic sequences, and they have a minimum size of 9 bases, preferably 18 bases, and more

preferably 36 bases. Finally, the invention is related to the use of a polynucleotide according to the invention as a sense or anti-sense nucleic acid sequence for controlling the expression of the  
5 corresponding protein product.

The non-labeled sequences of polynucleotides according to the invention may directly be used as a probe, a primer or an oligonucleotide; however the used sequences are generally labeled for obtaining usable  
10 sequences for many applications. The labeling of primers, probes, oligonucleotides according to the invention is achieved through radioactive elements or through non-radioactive molecules;  $^{32}\text{P}$ ,  $^{33}\text{P}$ ,  $^{35}\text{S}$ ,  $^3\text{H}$ , or  
15  $^{125}\text{I}$  may be mentioned among the used radioactive isotopes. The non-radioactive entities are selected from ligands such as biotin, avidin, streptavidin, dioxygenin, haptene, dyes, luminescent agents, such as radioluminescent, chemiluminescent, bioluminescent, fluorescent, phosphorescent agents.

20 The polynucleotides according to the invention may thus be used as a primer and/or a probe in methods notably implementing the PCR (polymerase chain reaction) technique (Erlich, 1989; Innis et al., 1990, and Rolfs et al., 1991). This technique requires the  
25 selection of pairs of oligonucleotidic primers framing the fragment which should be amplified. Reference may for example, be made to the technique described in the US Patent No. 4,683,202. The amplified fragments may be identified, for example after agarose gel or  
30 polyacrylamide electrophoresis or after a chromatographic technique like gel filtration or ion exchange chromatography. The specificity of the amplification may be controlled by molecular

hybridization by using as a probe, nucleotidic sequences of polynucleotides of the invention, plasmids containing these sequences or their amplification products. Amplified nucleotidic fragments may be used  
5 as reagents in hybridization reactions in order to demonstrate the presence, in a biological sample, of a target nucleic acid with a sequence complementary to that of said amplified nucleotidic fragments.

The invention is also directed to nucleotidic  
10 fragments which may be obtained through amplification by means of primers according to the invention.

Other techniques for amplifying the target nucleic acid may advantageously be used as an alternative to PCR (PCR-like) by means of a pair of primers for  
15 nucleotidic sequences according to the invention. It is understood that the term "PCR-like" designates all methods implementing direct or indirect reproductions of nucleic acid sequences, or else those in which the labeling system has been amplified, of course these  
20 techniques are known, generally this deals with DNA amplification by a polymerase; when the original sample is an RNA, a reverse transcription should be performed beforehand. Presently, there are very many methods which provide such amplification, such as for example,  
25 the SDA (Strand Displacement Amplification) technique (Walker *et al.*, 1992), the TAS (Transcription-based Amplification System) technique described by Kwok *et al.*, in 1989, the 3SR (Self-Sustained Sequence Replication) technique described by Guatelli *et al.*, in  
30 1990, the NASBA (Nucleic Acid Sequence Based Amplification) technique described by Kievitis *et al.*, in 1991, the TMA (Transcription Mediated Amplification) technique, the LCR (Ligase Chain Reaction) technique

described by Landegren *et al.*, in 1988, and enhanced by Barany *et al.*, in 1991, which uses a thermostable ligase, the RCR (Repair Chain Reaction) technique described by Segev in 1992, the CPR (Cycling Probe

5 Reaction) technique described by Duck *et al.*, in 1990, the Q-beta-relicase amplification technique described by Miele *et al.*, in 1983, and notably enhanced by Chu *et al.*, in 1986 and Lizardi *et al.*, in 1988, and then by Burg *et al.*, as well as Stone *et al.*, in 1996.

10 If the target polynucleotide is an RNA, for example a RNAm, a reverse transcriptase type enzyme will advantageously be used before implementing an amplification reaction with the primers according to the invention or before implementing a detection method

15 with probes of the invention, in order to obtain a DNAc from the RNA contained in the biological sample. The obtained DNAc will then be used as a target for the primers or the probes implemented in the detection or amplification method according to the invention.

20 The nucleotidic probes according to the invention, specifically hybridize with a DNA or RNA polynucleotide molecule according to the invention, more particularly with the sequence SEQ ID No.1 coding for the ECBP90 polypeptide, under strong stringency hybridization

25 conditions such as those given as an example earlier.

The hybridization technique may be used in different ways (Matthews *et al.*, 1988). The most general method consists of immobilizing the nucleic acid extracted from cells of different tissues or from

30 cells cultivated on a support (such as nitrocellulose, nylon, polystyrene) and of incubating, under well defined conditions, the immobilized target nucleic acid with the probe. After hybridization, the probe excess

is removed and the formed hybrid molecules are detected by the suitable method (measurement of radioactivity, fluorescence or enzyme activity related to the probe).

According to another embodiment of the nucleic probes, according to the invention, the latter may be used as a capture probe. In this case, a so-called "capture probe" is immobilized on a support and is used for capturing through specific hybridization, the target nucleic acid obtained from the biological sample to be tested and the target nucleic acid is then detected by a second probe, a so-called "detection probe", labeled with an easily detectable element.

In a preferred embodiment, the invention comprises the use of a sense or anti-sense oligonucleotide for controlling the expression of the corresponding protein product. Among the interesting nucleic acid fragments, anti-sense oligonucleotides i.e. those for which the structure provides an inhibition of the expression of the corresponding product, by hybridization with the target sequence, may be mentioned in particular. The sense oligonucleotides which, through interaction with the proteins involved in the control of the expression of the corresponding product which will induce either an inhibition, or an activation of this expression, should also be mentioned. The oligonucleotides according to the invention, have a minimum size of 9 bases, preferably 18 bases, and more preferably 36 bases.

The invention relates to a recombinant vector for cloning a polynucleotide according to the invention and/or for expressing a polypeptide according to the invention characterized in that, it contains a polynucleotide according to the invention, as described

earlier. The vector according to the invention, is characterized in that it includes components for the expression, possibly the secretion, of said sequences in a host cell. These vectors are useful for 5 transforming host cells in order to clone or express nucleotidic sequences of the invention. Particular vectors are for examples the vectors of plasmidic or viral origin. Among these vectors, those of the pGEX series (Pharmacia) for expression in bacteria or pSG5 10 (Stratagene, La Jolla, CA USA) are preferred for expression in a eukaryotic system.

The invention further comprises host cells, notably eukaryotic and prokaryotic cells, characterized in that they are transformed with vectors according to 15 the invention. Preferably, the host cells are transformed under conditions allowing a recombinant polypeptide according to the invention to be expressed. The cell host may be selected from bacterial cells (Olins and Lee, 1993), but also from yeast cells 20 (Buckholz, 1993), as well as animal cells, in particular mammal cell cultures (Edwards and Aruffo, 1993), but also insect cells wherein methods implementing baculoviruses for example may be used (Luckow, 1993). These cells may be obtained by 25 introducing into the host cells a nucleotidic sequence inserted in a vector such as defined above, and then by growing said cells under conditions providing replication and/or expression of the transfected nucleotidic sequence.

30 The invention also relates to a method for preparing a polypeptide, characterized in that it implements a vector according to the invention. More specifically, the invention relates to a method for

preparing a recombinant polypeptide characterized in that the transformed cells according to the invention are grown under conditions providing expression of said recombinant polypeptide and in that said recombinant 5 polypeptide is recovered.

The polypeptide according to the invention may be obtained according to a method of the invention, and according to production techniques for recombinant polypeptides, known to one skilled in the art. The 10 present invention therefore relates to the recombinant polypeptide which may be obtained by the method shown above. In this case, the nucleic acid sequence used is placed under the control of signals providing its expression in a cell host. An efficient production 15 system for a recombinant polypeptide requires the availability of a vector, for example of plasmidic or viral origin and of a compatible host cell. The vector should include a promoter, signals for initiating and terminating the translation, as well as suitable 20 regions for controlling the transcription. It should be able to be maintained in the cell stably and may optionally have particular signals specifying the secretion of the translated polypeptide. These different control signals are selected depending on the 25 used host cell. For this purpose, the nucleic acid sequences according to the invention may be inserted in autonomous replication vectors inside the selected host or integrative vectors of the selected host. Such vectors are prepared according to methods currently 30 used by one skilled in the art and the resulting clones may be introduced into a suitable host by standard methods such as for example transfection with calcium phosphate precipitation, lipofection, electroporation,

thermal shock.

The recombinant polypeptides obtained as indicated above, may both exist in the glycosylated and non-glycosylated form and may have the natural tertiary  
5 structure or not.

The polypeptides obtained through chemical synthesis and which may include non-natural amino acids corresponding to said recombinant polypeptides, are also comprised in the invention. The peptides according  
10 to the invention may also be prepared by conventional techniques, in the field of peptide synthesis. This synthesis may be carried out in a homogenous solution or in the solid phase.

The methods used for purifying recombinant  
15 polypeptides are well known to one skilled in the art. The recombinant polypeptide may be purified from lysats and cell extracts, from the supernatant of the culture medium, by methods either used individually or in combination, such as fractionation, chromatography  
20 methods, immuno-affinity techniques by means of specific mono- or polyclonal antibodies, etc.

A preferred alternative consists of producing a recombinant polypeptide fusioned to a "carrier" protein (chimeric protein). The advantage of this system is  
25 that it provides stabilization and a reduction in the proteolysis of the recombinant product, an increase in the solubility during renaturation *in vitro* and/or a simplification of the purification when the fusion partner has an affinity for a specific ligand.

30 The invention also relates to a monoclonal or polyclonal antibody and to its fragments, characterized in that they specifically bind a polypeptide according to the invention. Chimeric antibodies, humanized

antibodies and simple chain antibodies are also part of the invention. Antibody fragments according to the invention are preferably Fab or F(ab')<sub>2</sub> fragments.

The polypeptides according to the invention allow 5 monoclonal or polyclonal antibodies to be prepared. Advantageously, monoclonal antibodies may be prepared from hybridomas according to the technique described by Kohler and Milstein in 1975. The inventors use this 10 technique for obtaining a hybridoma producing a new highly specific monoclonal antibody of an epitope of protein ICBP90.

Polyclonal antibodies may be prepared, for example, by immunizing an animal, for example a mouse, with a polypeptide according to the invention 15 associated with an adjuvant from the immune response, and then by purifying the specific antibodies contained in the serum of the immunized animals on an affinity column on which is fixed beforehand the polypeptide which has been used as an antigen. The polyclonal 20 antibodies according to the invention may also be prepared by purification on an affinity column, on which a polypeptide according to the invention has been immobilized beforehand.

The invention also relates to a specific 25 monoclonal antibody of the human ICBP90 protein and capable of inhibiting interaction between ICBP90 and the DNA sequence onto which protein ICBP90 specifically binds. According to another embodiment, the monoclonal antibody according to the invention and specific to the 30 human ICBP90 protein is capable of inhibiting the interaction between ICBP90 and the proteins with which interacts ICBP90, said proteins preferably being ICBP90 itself, or proteins from the transcriptional complex.

It is understood that the term "proteins from the transcriptional complex" designates all proteins participating in the transcription reaction whether this happens in the initiation, elongation, or  
5 termination of the transcription.

The antibodies of the invention may also be labeled in the same way as described earlier for the nucleic probes of the invention, and preferably with an enzymatic, fluorescent or radioactive type labeling.

10 Moreover, in addition to their use for purifying polypeptides, the antibodies of the invention, in particular the monoclonal antibodies, may also be used for detecting these polypeptides in a biological sample.

15 They thus form a means for analyzing the expression of the polypeptide according to the invention, for example through immunofluorescence, labeling with gold, enzymatic immunoconjugates.

More generally, the antibodies of the invention  
20 may advantageously be implemented in any situation where the expression of a polypeptide according to the invention needs to be observed, and more particularly in immunocytochemistry, in immunohistochemistry, or in Western blotting experiments.

25 Thus, the invention relates to a method for detecting and/or dosing a polypeptide according to the invention, in a biological sample, characterized in that it comprises the following steps for bringing the biological sample into contact with antibodies  
30 according to the invention and then for detecting the formed antigen-antibody complex. This method may be used in immunocytochemistry for cell localization of the polypeptide according to the invention and in

immunohistochemistry for assessing cell proliferation.

A kit for detecting and/or dosing a polypeptide according to the invention in a biological sample, is also within the scope of the invention, characterized  
5 in that it comprises the following components: (i) a monoclonal or polyclonal antibody such as described earlier; (ii) if necessary, the reagents for forming the favorable medium for the immunological reaction;  
10 (iii) the reagents for detecting the antigen-antibody complexes produced by the immunological reaction. This kit is notably useful for conducting Western blotting experiments; with the latter, control of the expression of the polypeptide according to the invention may be investigated starting with tissues or cells. This kit  
15 is also useful for immunoprecipitation experiments in order to notably detect proteins which interact with the polypeptide according to the invention.

Any conventional procedure may be implemented for carrying out such a detection and/or dosage. As an  
20 example, a preferred method involves immunoenzymatic processes according to the immunofluorescence or radioimmunological (RIA) ELISA technique or equivalent.

The invention also comprises a method for detecting and/or dosing a nucleic acid according to the  
25 invention, in a biological sample, characterized in that it includes the following steps: (i) isolation of the DNA from the biological sample to be analyzed, or obtaining a DNAc from the RNA of a biological sample;  
(ii) specific amplification of the DNA coding for the  
30 polypeptide according to the invention by means of primers; (iii) analysis of the amplification products.

The invention further comprises a kit for detecting and/or dosing a nucleic acid according to the

invention, in a biological sample, characterized in that it comprises the following components: (i) a pair of nucleic primers according to the invention, (ii) the required reagents for carrying out a DNA amplification reaction and optionally (iii) a component for checking the sequence of the amplified fragment, more particularly a probe according to the invention.

The invention also comprises a method for detecting and/or dosing a nucleic acid according to the invention, in a biological sample, characterized in that it includes the following steps: (i) bringing a probe according to the invention into contact with a biological sample; (ii) detecting and/or dosing the hybrid formed between said probe and the DNA of the biological sample.

The invention also comprises a kit for detecting and/or dosing a nucleic acid according to the invention, in a biological sample, characterized in that it comprises the following components: (i) a probe according to the invention, (ii) the reagents required for implementing a hybridization reaction and if necessary, (iii) a pair of primers according to the invention, as well as the reagents required for an DNA amplification reaction.

The invention particularly relates to methods according to the invention and described above, for detecting and diagnosing cell proliferation, and more particularly cell proliferation of cancerous origin.

The invention also relates to a method for screening ligands able to affect the transcriptional activity of a gene, the promoter of which includes CCAAT and/or inverted CCAAT boxes capable of binding a polypeptide according to the invention, said method

being characterized in that it includes the following steps for bringing into contact said polypeptide and one or several potential ligands in the presence of reagents required for implementing a transcription or 5 detection reaction and/or a reaction for measuring transcriptional activity. One of the objects of the invention is also to provide a kit or package for screening ligands able to affect the transcriptional activity of a gene, the promoter of which includes 10 CCAAT and/or inverted CCAAT boxes capable of binding a polypeptide according to the invention characterized in that it comprises the following components: (i) a polypeptide according to the invention; (ii) a ligand; (iii) the reagents required for implementing a 15 transcription reaction.

It is understood that the term "ligand" defines all compounds able to interact with the polypeptide according to the invention, in order to form a complex able to affect the transcriptional activity, i.e. to 20 increase, reduce, modulate or cancel the transcription of a gene under the control of a promoter containing a DNA sequence to which binds the polypeptide of the invention.

It is also understood that the term "ligand" 25 defines any compound able to bind to the binding DNA sequence for the polypeptide according to the invention. Such a ligand forms a competitive inhibitor of the polypeptide according to the invention for its binding to the DNA sequence.

30 Preferably, the biological sample according to the invention in which detection and dosage is performed, consists of a body fluid, for example human or animal serum, blood, saliva, lung mucus, or biopsies.

According to another aspect, the invention relates to a compound characterized in that it is selected from an antibody, a polypeptide, a ligand, a polynucleotide, an oligonucleotide, or a vector according to the 5 invention as a drug, and notably as active ingredients of a drug: these compounds preferably will be in soluble form, associated with a pharmaceutically acceptable carrier. It is understood that the term "pharmaceutically acceptable carrier" designates any 10 type of carrier usually used in preparing injectable compositions, i.e. a diluent, a suspension agent, such as an isotonic or buffered saline solution. Preferably, these compounds will be administered systemically, in particular intravenously, intramuscularly, 15 intradermally, or orally. Their modes of administration, dosages and optimal dosage forms may be determined according to the criteria generally considered in establishing a suitable treatment for a patient as for example, the age or body weight of the 20 patient, the seriousness of his/her general condition, tolerance to the treatment and ascertained secondary effects, etc.

According to a preferred embodiment, the compound according to the invention is used as a drug for the 25 prevention and/or the treatment of cancer.

The compound according to the invention is used for the preparation of a pharmaceutic designed to modulate, raise, or diminish cellular proliferation.

The invention also has at its foundation a 30 pharmaceutical composition that can act in the preventive and curative treatment of cancer and is characterised by a therapeutically effective quantity of an active compound and a pharmaceutically acceptable

excipient. Using the preferred method of synthesis, this pharmaceutical composition contains antibodies that serve as targeting agents; those antibodies are conjugated to at least one agent selected from among 5 antiproliferative, antineoplastic, or cytotoxic agents. These agents are either radioisotopes or non-isotopic substances. The conjugation of antibodies contained in the present invention with antiproliferative, antineoplastic, or cytotoxic agents can be utilized for 10 arresting the development of cancers and for inducing regression and even elimination of tumoural masses. Preferably, the antibody or the antibody fragment conjugated to the agent is administered to the cancer patient and delivered to tumour sites by oral or 15 parenteral route through a pharmaceutically acceptable transporting liquid, such as saline. Alternatively, a solution or suspension of antibody and antibody fragment conjugated to an agent can be perfused directly into the tissue of a malignant epithelial 20 cancer, a method used by preference when the cancer has not metastasized.

For therapeutic use, the preferred radioisotopes, conjugated to monoclonal antibodies, are gamma emitters, the most effective being iodine<sup>131</sup>, yttrium<sup>90</sup>, 25 gold<sup>199</sup>, palladium<sup>100</sup>, copper<sup>67</sup>, bismuth<sup>217</sup>, and antimony<sup>211</sup>. Alpha and beta emitting radioisotopes can also be employed for therapy. Non-isotopic substances conjugated to monoclonal antibodies and used for therapy are abundant and varied; for example: (i) 30 antimetabolites, such as anti-folate agents like methotrexate, (ii) purine and pyrimidine analogues (mercaptopurine, fluorouracil, 5-azacytidine, (iii) antibiotics, (iv) lectins (ricin, abrin) and (iv)

bacterial toxins (diphtheria toxin).

The antibodies of the invention can also be used as targeting agents to target cytotoxic cells, such as human T cells, monocytes or NK cells present or not at 5 a metastasised tumour site. Antibodies can attach to cytotoxic cells via the Fc receptor situated at the surface of these cells or via an intermediary antibody that has a double specificity. Such bi-specific antibodies for the targeting of cancerous cells can be 10 produced by fusing an immune cell producing the antibody of the present invention or a hybridoma of the present invention with a cell producing an antibody directed against the targeted cytotoxic cell. Bi-specific antibodies can equally be produced by 15 chemically coupling two antibodies having the desired specificity. The antibodies of this invention also permit the targeting of carriers bearing antiproliferative, antineoplastic, or cytotoxic agents to the site of the tumor or metastatic tumor. By 20 carriers we are referring to liposomes and viral particles. In certain cases, it's possible to predetermine the target elements to assure a specific expression in certain tissues or cells and limit the expression zones of the polypeptides of this invention.

25 The invention also concern a product comprising at least a compound of the invention, and at least an anticancerous agent as a combination product for a simultaneous, separated or delayed use over the time.

30 In summary, the invention concerns a composition for the detection, localisation, and imaging of cancers, using an antibody that is tagged directly or indirectly by a marker whose signal is generated by

radioactive or non-isotopic substances as defined above. The invention also has as objective the localisation and imaging of cancers, including (i) the stages of dispersion after parenteral injection into a 5 human of a composition based on the invention; (ii) the accumulation of tagged antibody, after an adequate time period, at the vicinity of cancer cells, then the penetration of those cells by the tagged antibody without significantly affecting normal cells; (iii) the 10 detection of a signal using an appropriate signal detector; and (iv) the conversion of the detected signal to an image of the cancerous cells.

Other characteristics and advantages of the invention are discussed after this description 15 accompanied by the examples below. In the examples, we will refer to the following figures.

Figure 1: Expression de la protein ICBP90 in HeLa cells (tumour cells) and in pulmonary fibroblasts in primary 20 culture (non-tumoral cells).

The detection of the endogenous protein, ICBP90, was carried out on total protein extracts from confluent (lane 1) and proliferating (lane 2) HeLa cells and on total protein extracts from primary 25 cultures of human pulmonary fibroblasts at confluence (lane 3) and in proliferation (lane 4). After migration in a polyacrylamide gel in the presence of 8% SDS, the proteins were transferred to nitrocellulose membranes by electrotransfer. The revelation of the protein was 30 performed using antibody 1RC1C-10 diluted to 1/4000 (initial concentration 2 mg/ml) and a secondary antibody coupled to alkaline phosphatase and directed against the heavy chains of mouse antibodies. In the

lanes corresponding to extracts from HeLa cells, there is a major band at 97 kDa; for proliferating HeLa cells, supplementary bands of sizes less than 97 kDa appear (lane 2). In confluent human pulmonary fibroblasts, the endogenous protein is not expressed (lane 3), while the protein does appear when the cells begin to proliferate (lane 4). These observations suggest that the endogenous ICBP90 protein is a marker of cellular proliferation for normal cells (fibroblasts), whereas for tumour cells, it is a marker regardless of the cellular stage.

Figure 2: Immunoprecipitation of the endogenous protein

Immunoprecipitation was carried out on total protein extracts from MOLT-4 cells. 1RC1C-10 antibodies were attached to the protein beads of G-Sepharose, then put into contact with protein extract for 2 hours at room temperature. After washing, the bead/1RC1C-10/protein complexes were precipitated by centrifugation and analysed by migration in a 8% polyacrylamide gel in the presence of SDS. They were then transferred to nitrocellulose membranes for revelation of the proteins as indicated in figure 1. A unique band appears at 97 kDa, as well as a band of 45 kDa corresponding to the heavy chain of 1RC1C-10.

Figure 3: Nuclear localisation of the endogenous protein

We used HeLa cells to examine the endogenous expression of the protein ICBP90 *in situ* employing 1RC1C-10 antibody and a secondary anti-mouse antibody coupled to fluorochrome CY3. The fluorescent marker localises exclusively in the nucleus. The nucleolus and

the cytoplasm are not labelled.

Figure 4: Expression of endogenous ICBP59 in proliferating cells

We observed endogenous protein in paraffin sections of human appendix. After deparaffinization and pre-treatment by heat in acid buffer (unmasking of antigenic sites), the sections were incubated for 16 hours with 1RC1C-10 antibodies diluted 1/10000 (initial concentration of 2 mg/ml). Revelation was performed by adding biotinylated secondary antibody, and then incubating with streptavidine-peroxidase complex. A counter-staining of nuclei by Harris' haematoxylin was also carried out. The labelling by 1RC1C-10 is localised essentially in zones of cellular proliferation. The labelled cells are found in glandular crypts (GC), as well as germinative zones (ger).

Figure 5: Expression of ICBP-59 in diverse human tissues

We evaluated the level of expression of mRNA corresponding to ICBP59 in 50 different human tissues using an RNA dot blot. The blot was hybridised for 16 hours at 68°C with a cDNA (32P) radioactive probe of 679 bp in ExpressHyb (Clontech) hybridisation solution. After washing several times, we revealed the protein by autoradiography (one week exposure at 80°C). The tissues demonstrating the highest expression level were foetal and adult thymus, as well as adult bone marrow and foetal liver.

Figure 6: Nucleotide sequence of ICBP90

cDNA coding for ICBP90 measures 2379 bp. The portions of sequence indicated in bold are those that do not appear in the human EST database (human dbest).

- 5 The other sequences exist in diverse EST:

From 1 to 325: EST n° AI083773,

From 367 to 865 EST n° AA811055.

From 940 to 1857 EST n° AA488755, EST n° AA129794  
and EST n° AA354253.

10

Figure 7: Protein sequence of ICBP90

The amino acid sequence of ICBP90 was deduced by translation of the nucleotide sequence from figure 6.

ICBP90 is composed of 793 residues and has a

- 15 theoretical molecular weight of 89,758 kDa. The pKi is  
7.7. The amino acids indicated in grey correspond to  
ICBP-59.

EXAMPLE 1: EVIDENCE OF A NEW BINDING PROTEIN FOR THE  
20 ICB SEQUENCE

1.1 Reporter construction for the screening of the library

The simple hybrid system is a powerful technique for detecting, *in vivo*, in yeast the interaction of proteins with specific DNA sequences when screening cDNA libraries. This technique allows you to evaluate directly cDNA corresponding to the protein to be linked. Several studies using this technique resulted in the identification of novel proteins. The protocols 25 are well described by Inouye *et al.* (1994) and Wang and Reed (1993).

Briefly, the following oligonucleotides have been synthesized:

5' -AATTCGATTGGTTCTGATTGGTCTGATTGGTCTT-3' and 5' -  
CTAGAAGAAC**CAATCAGAAC**CAATCAGAAC**CAATCG****-3'. These  
nucleotides were then hybridised. According to the  
documentation of the manufacturer (Clontech, Palo Alto,  
5 CA), the reporter construct targeted possesses three  
copies in tandem of the ICB2 sequence (ICB2X3). As  
mentioned above, one copy of ICB2 is underscored and  
the CCAAT sequences are in bold. To determine the  
specificity of protein binding to the ICB box, the  
10 following oligonucleotides, containing three copies in  
tandem of the GC1 box (GC1X3), also present in the  
promoter, have been synthesized and hybridised:**

5' -AATTCGGGCGGGGCCGGGGCGGGCCCGGGGCT-3'

5' -CTAGAGCCCCGCCCGGGCCCCGCCCGGGCCCCCGG-3'

15 The resulting target DNA fragments were cloned  
into the polylinker of the pHISi-1 integrative plasmid  
(Clontech) by cohesive-end ligation to the plasmid's  
XbaI-EcoRI site, upstream of the minimal promoter of  
the gene, *his3*. The yeast strain, YM4271 (Clontech),  
20 was used for the transformation. Transformed colonies  
of yeast containing the plasmid integrated in their  
genomes were selected by cultivating the yeast in  
synthetic dropout medium lacking histidine. We isolated  
two colonies: one for ICB2 and the other for the GC1  
25 box.

### 1.2 Screening the library

A cDNA library from the Jurkat cell line, cloned  
into the EcoRI site of the polylinker downstream of  
GAL4-AD of the pGAD10 vector (Clontech), was used for  
30 screening according to the manufacturer's instructions.  
Positive clones were selected, and then cultivated in  
selective medium depleted of histidine and leucine. The  
plasmid DNA of the clones was recuperated and

introduced by electroporation into the bacterial *Escherichia coli* strain, XL1-blue. The sequencing of the inserts were carried out on a matrix of plasmid DNA purified from a 1.5 ml culture using a mini preparation 5 kit (Bio-Rad, Hercules, CA, USA). A cDNA library of human thymus cloned in λgt10 (Clontech) was screened by plaque hybridisation to recuperate a cDNA coding for the N-terminal part of the protein.

### 1.3 Discovery of ICBP-59

10 The cDNA from four clones selected using the simple hybrid system was sequenced, then analysed employing a digital database (Genbank, EMBL, PDB, Swissprot) to determine the nature of the coded proteins. Two of the clones correspond to ribosomal 15 proteins (hRS12 and hRS4), one to a serine-threonine kinase (STPLK-1), and the fourth to a human protein having theoretical molecular weight of 59 kDa (calculated from the translated sequence) that does not appear in the database.

20 The cDNA coding for hRS4, hRS12, and ICBP-59, and obtained by EcoRI digestion of positive clones in the pGAD10 vector, were cloned into the EcoRI site of the expression vector pGEX-4T-1 (Pharmacia). The recombinant DNA was then transformed in an adapted 25 mouse *Escherichia coli* strain (BL21). We then used a 500 ml culture of a selected clone once the culture reached a density of 0.5. The overexpression of proteins under study was induced by incubation with IPTG (1 mM) for 2 hours at 37°C. The pGEX-4T-1 vector 30 makes possible the recovery of large quantities of proteins fused to glutathione S-transferase (GST). The GST fusion proteins are then purified using Sepharose beads coupled to glutathione (Pharmacia) followed by

overnight cleavage with thrombin (0.05 U/ml) at 4° C (Pharmacia).

To test the ability of the 59 kDa protein to bind specifically to the ICB1 and/or ICB2 boxes, three 5 tandem copies of ICB2 (ICB2X3, sequences described above) were labelled at the terminal end with  $^{32}$  P phosphore using the T4 polynucleotide kinase (New England Biolabs) and [ $\gamma^{32}$ P]ATP (160 mCi/mmol, ICN Irvine, CA, USA). To examine the specificity of the 10 binding, oligonucleotides containing only one copy of the CCAAT box were synthesized:

ICB1: 5'-AGTCAGGGATTGGCTGGTCTG-';

5'-CAGACCAG**CCAAT**CCCTGACT-3'

ICB2: 5'-AAGCTACG**ATTGGTTCTTCTG**-3';

15 5'-CAGAAGAAC**CCAAT**CGTAGCTT-3'.

The ICBP-59 protein (1  $\mu$ g) was incubated with 1 ng of oligonucleotide and labelled at its terminal end by phosphorous  $^{32}$ P in 12% glycerol, 12 mM HEPES-NaOH (pH 7.9), 60 mM KCl, 4 mM Tris-HCl (pH 7.9), 100 ng BSA, 20 0.6 mM DTT, and 100 ng poly(dI/dC) in 20  $\mu$ l (Inouye et al., 1994). After a 30-minutes incubation at room temperature, the reaction mix was loaded in 6% polyacrylamide gels. In competition experiments, the quantity indicated of non-labelled oligonucleotides 25 were added to the reaction mix 10 minutes before the addition of proteins. To examine the binding properties of ICBP90 with regard to the ICB2 box, we used the same protocol except that labelled oligonucleotide contained only one copy of the CCAAT sequence as described below:

30 ICB2: 5'-ATAAAGGCAAGCTACG**ATTGGTTCTTCTGGACGGAGAC**-3'

5'-GTCTCCGTCCAGAAGAAC**CCAAT**CGTAGCTTGCCTTTAT-3'

Binding specificity was studied using a non-labelled nucleotide containing a GC box of the human

topoisomerase II $\alpha$  promoter:

5'-GAATTCGAGGGTAAAGGGGCGGGTTGAGGCAGATGCCA-3'

5'-TGGCATCTGCCTCAACCCCGCCCCTTTACCCCTCGAATTC-3'.

These gel retardation experiments in acrylamide gels has given us evidence that the new 59 kDa human protein can specifically bind an ICB DNA sequence. We have called this protein ICBP-59 (for inverted CCAAT Box Binding Protein of 59 kDa).

10 EXAMPLE 2: CHARACTERISATION OF THE ICBP90 PROTEIN

2.1. Synthesis of antibodies

Mouse monoclonal antibodies were synthesized in our laboratory by injection of ICBP-59 protein using traditional methods (Brou *et al.*, 1993); the protein was purified beforehand by a fusion GST system. Two monoclonal antibodies from 1RC1C-10 and 1RC1H-12 were selected for their ability to detect the ICBP-59 endogenous protein; their specificity was demonstrated in both Western blotting and immunocytochemistry experiments. Before use, the antibodies were purified on a DEAE-cellulose column (DE52, Whatmann) from ascites fluid.

2.2 Detection of the endogenous protein by Western blotting

To detect endogenous ICBP-59 protein, we first used 1RC1C-10 in a Western blot (0.4  $\mu$ g/ml 1RC1C-10 monoclonal antibodies) of nuclear extracts from confluent and proliferating HeLa cells (Figure 1). COS-30 1 and HeLa cells were cultivated as previously described (Brou *et al.*, 1993; Gaub *et al.*, 1998; Rochette-Egly *et al.*, 1997). MOLT-4 cells were cultured in 100% air in RPMI supplemented with 10% foetal calf

serum. Primary cultures of human pulmonary fibroblasts were prepared and grown in DMEM/F12 as previously described (Kassel et al., 1998). We purchased nuclear extracts of Jurkat cells from Sigma, while we prepared 5 the extracts from MOLT-4 and HL60 as already described in the literature (Lavie et al. 1999). Proliferating HeLa cells and human pulmonary fibroblasts were obtained by depleting their culture media of serum for 30 hours, then reintroducing foetal calf serum to a 10 concentration of 10% (v/v) for 16 hours. Proliferation was arrested when the cells reached 60 to 70% confluence. Cell cultures stopped at confluence (100% confluence) were prepared in the same way, omitting the serum depletion step. For these two types of cells, 15 total cellular extracts were prepared by first harvesting the cells in PBS (phosphate buffered saline), then sonicating them. Immunotransfer experiments on total cell lysates and nuclear extracts involved loading the material on 8% SDS polyacrylamide 20 gels and performing a one-dimensional electrophoresis. The proteins were transferred to nitrocellulose membranes that had been blocked with 10% blocking reagent (Roche Molecular Biochemical, Mannheim, Germany). They were then incubated with 1RC1C-10 25 purified monoclonal antibodies at a concentration of 0.5 µg/ml. A sheep anti-mouse antibody coupled to alkaline phosphatase (fragments Fab, Roche Molecular Biochemicals) was used at a 1/2500 dilution. The signals were detected using 4-nitro blue tetrazolium 5- 30 bromo-4-chloro-3-indolyl-phosphate chloride as substrate.

These experiments show that the endogenous protein has a molecular weight of approximately 97 kDa.

Moreover, we observed that the form of the protein varies according to its tumoural or non-tumoural nature, as well as the state of confluence or proliferation of the cells. For example, in the lanes 5 corresponding to extracts from HeLa cells, there is a major band at 97 kDa; for proliferating HeLa cells, supplementary bands of sizes inferior to 97 kDa appear (lane 2). In confluent human pulmonary fibroblasts, the endogenous protein is not expressed and appears when 10 the cells begin to proliferate (lane 4). These observations suggest that the endogenous protein ICBP90 is a marker of cellular proliferation in normal cells (fibroblasts), while, in tumour cells, it would be a marker at any cellular stage.

15 The use of monoclonal antibodies in immunoprecipitation experiments on nuclear protein extracts, followed by Western blotting, further puts in evidence the presence of a 97 kDa protein (Figure 2).

20 The results obtained from Western blotting, for both nuclear protein extracts and immunoprecipitations, show that the 59 kDa protein isolated by the simple hybrid system constitutes a fragment of the corresponding human endogenous protein, in this case, the C-terminal fragment from residue D263. It was, 25 therefore, necessary for us to undertake a new screening of the cDNA library.

### 2.3. Multiple Human Tissues RNA Dot Blot Analysis

In order to choose a library providing us with the best possible chance to isolate the complete 30 protein, we wanted to identify a human tissue expressing the corresponding messenger RNA (mRNA). With a  $^{32}P$  labelled cDNA probe covering part of the ICBP59 sequence, we tested the mRNA expression of interest in

50 different human tissues against a RNA Dot Blot. Briefly, a 678 base pair probe corresponding to the ICBP90 amino acids sequence 269 to 500 was synthesized by PCR using Taq polymerase (Sigma, St Louis, MO, USA).

- 5 The probe labelled by random priming using dCTP -  $\alpha$  32P was purified on Sephadex G50 columns (Pharmacie, Uppsala, Sweden).

A multiple organ RNA Dot Blot containing poly(A); RNA from 50 different human tissues was hybridised for 10 20 hours under strong stringency conditions in an ExpressHyb environment (Clontech) at 68° C with a 32P labelled probe. High stringency washing was completed in 0.1 x SSC, 0.1% SDS at 68° C (De Vries et al., 1996).

- 15 The results obtained (fig. 5) show that tissues expressing most strongly the ICBP-59 protein mRNA are adult and foetal thymus, as well as adult bone marrow and foetal liver. Therefore, to isolate the whole protein, we choose an adult thymus cDNA library.

20 2.4. Library Screening and ICBP90 Cloning

The bank screening permitted us to obtain several clones of about 4000 base pairs (bp) containing a 2379 bp open reading frame (Fig. 6). This sequence codes for a 793 amino acid protein (Fig.7), which theoretical 25 molecular weight (calculated from the translated sequence) is 89.758 kDa. We called this protein ICBP90 (for Inverted CCAAT Box Binding Protein of 90 kDa) by analogy to the initial 59 kDa protein name.

The ICBP90 cDNA (2379 bp) was synthesized by PCR 30 using Deep Vent DNA polymerase (New England Biolabs, Beverly, MA, USA) and oligonucleotides used during this PCR reaction were near the EcoRI site. The product of the reaction was thereafter sub-cloned in a pGEX-4T-1

vector (Pharmacie) for the GST fusion protein expression in BL21. The over expression was induced by IPTG (1mM) for 4h at 25°C. The ICBP90 protein was then purified.

5        2.5. Immunocytochemistry and Immunohistochemistry.

The direct observation of the ICBP90 protein on cells and tissues was also carried out.

COS-1 cells were transfected as describes previously (Brou *et al.*, 1993; Gaub *et al.*, 1998) with 10 the pSG5 vector (Stratagene, La Jolla, CA) in which the ICBP90 cDNA (2379 bp) was sub-cloned in the EcoRI restriction site. The cDNA was synthesized by polymerisation chain reaction (PCR) using Deep Vent polymerase (New England Biolabs) and the 15 oligonucleotides flanking the EcoRI restriction site. Plasmidic construction was verified by sequencing. The immunolabelling of the transfected HeLas and COS-1 cells was achieved as described previously (Brou *et al.*, 1993) with 1RC1C-10 and 1 RC1H-12 monoclonal 20 antibodies, respectively. An indirect labelling with ICBP90 immunoperoxidase and II $\alpha$  topoisomerase was achieved as described previously (Rio *et al.*, 1987, Devys *et al.*, 1993). Human appendices were embedded in paraffin and fixed in 10% buffered formalin (Sigma). 25 Serial sections (3 gm) were incubated overnight at room temperature with 1 RC1C-10 antibody and with II $\alpha$  anti-topoisomerase antibody (NeoMarkers, Union City, CA, USA). Antibodies bound in a specific manner are visualized through a complex using streptavidine biotin 30 (LAB/LSAB method, Dako LSAB2 System kit; DAKO, Carpinteria, CA, USA).

In immunocytochemistry the 1RC1C-10 antibody labels the HeLa cells nucleus whereas the nucleolus and

the whole cytoplasm are not labelled (Fig. 3). In immunohistochemistry, paraffin-embedded human appendix sections show labelling essentially localized in cellular proliferation zones (Fig. 4). Indeed, the 5 labelled cells were located in the glandular crypts (CG) as well as in the germinative zones (Ger). An identical labelling is obtained when using an II $\alpha$  anti-topoisomerase antibody, an enzyme essentially expressed in proliferating cells (results non illustrated).

10        2.6. BLAST Research and Domain Prediction

Studies about on-line BLAST have been carried out based on information from the National Centre for Biotechnology Information at the National Institute of Health (Bethesda, MD, USA). SCANPROSITE and 15 PROFILESCANS were used for protein analysis (Infobiogen, Villejuif, France).

ICBP90 includes a "ubiquitin like" domain in its first 80 amino acids, two sites of potential nuclear localizations in its C terminal and two zinc finger-20 like domains, one of which could be implicated in the DNA linkage and the other in protein-protein interactions. Several potential phosphorylation sites by protein kinase C, the casein kinase II, as well as by a tyrosine kinase, were also present.

25        ICBP90 production and purification using the GST fusion system (same procedure as for ICBP-59) permitted to finally test the complete protein ability to link the ICB type DNA sequences. Its behaviour is identical from top to bottom to that observed for ICBP-59.

30        Finally, we isolated a new human protein that we called ICBP90 for the reasons evoked above. Its theoretical molecular weight is 89.758 kDa and its apparent molecular weight on acrylamide gel is 97 kDa.

This protein is not only localized exclusively in human cell nuclei, but it also presents the ability to bind specifically DNA sequences, in this case CCAAT type sequences. For these reasons, we think that ICBP90 has  
5 the possibility to modulate the expression of genes which promoter is provided with CCAAT boxes, possibly in reversed position (ICB). The gene of the human topoisomerase IIa we are especially interested in, and which includes five ICB sequences in its promoter,  
10 seems to be one of ICBP90 privileged targets.

These experiences allowed to bring to light the 1RC1C-10 antibody remarkable features, which only labels proliferating cells in the case of non cancerous cells; it labels both proliferating and quiescent  
15 cancerous cells; it is usable with 4 different techniques (Western blotting, Immunocytochemistry, immunohistology, immunoprecipitation); it has a very good affinity and allow for the use of 1/150,000 dilution in immunohistochemistry (13 ng/ml); finally,  
20 its use generates nearly no background noise.

Future applications of 1RC1C-10 are primarily for diagnostic and basic research. For anatomo-pathologic diagnostics for instance, it would be quite possible to assess the proliferative state of a given cancerous  
25 tissue. Regarding basic research, investigations are in progress in our laboratory in order to determine the exact contribution of ICBP90 to proliferation mechanisms in normal and cancerous cells. However, the use of antibodies will be required to study ICBP90  
30 expression as a function of the cellular cycle, of its precise nuclear localization and of its interaction with other cellular proteins.

At the moment we haven't study the expression of

ICBP90 with regards to cellular cycle. Nevertheless, in the case where cancerous cell lineages are confluent or when they are not proliferating, we can detect significant differences of ICBP90 expression (Fig. 1) 5 at least with regard to the 97 kDa form. On the other hand, in the non-cancerous confluent cells (human bronchial smooth muscular cells) the ICBP90 expression is hard to detect (results not illustrated). This was confirmed with histological sections where no quiescent 10 cells were labelled by the antibody. It is therefore possible that ICBP90 is expressed whatever the cellular cycle phase in cancerous cells whereas its expression would vary according to each phase in non-cancerous cells. Therefore, this makes the use of the antibody 15 extremely interesting, as, contrary to other cellular proliferation label such as Ki-67, topoisomerase II $\alpha$ , cycline E and cycline B1, we would have at our disposition a label for cancerous tissue proliferating cells that would not depend on the cellular cycle 20 phase. Indeed, the end of the S phase is characterized by a very weak Ki-67expression, cycline E labels cells at the end of phase G1 up to the middle of phase S, and cycline B1 labels cells in phase G2/M (for a review, see Darzynkiewicz *et al.*, 1994). Moreover, it has been 25 shown that PCNA (Proliferating Cell Nuclear Antigen) overestimates the number of proliferating cells in some types of tissues (Roskell and Biddolph, 1999).

ICBP90 plays an important role in cellular proliferation by regulating the expression of genes 30 such as those for topoisomerase. II $\alpha$ . Different strategies aiming at blocking the action of this protein must allow modifying cellular proliferation. Anyway, the uses of the 1RC1C-10 antibody as well as of

peptides mimicking the ADN/ICBP90 interaction without generating subsequent physiological effect constitute an interesting possibility. The design of its peptides would be directly inspired from the ICBP90 protein  
5 sequence we described. A truncated form corresponding to ICBP59 could be one of the first candidates, for instance.

The simple blockage of ICBP90 expression in order to completely eliminate its influence on genes and, by  
10 extension, on cellular proliferation can be considered; it could be carried out either by a classic approach such as obtaining inhibitors of the protein, or by a more modern approach corresponding to the interference technique with-double strand RNA (RNA interference or  
15 RNAi) as describes recently by Kennerdell & Carthew (1998).

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## SEQUENCE LISTING

&lt;110&gt; ADEREGEM

&lt;120&gt; ICBP90 POLYPEPTIDE AND ITS FRAGMENTS AND POLYNUCLEOTIDES CODING FOR SAID POLYPEPTIDES AND APPLICATIONS FOR DIAGNOSING AND TREATING CANCER

&lt;130&gt; D18243

&lt;160&gt; 11

&lt;170&gt; PatentIn Ver. 2.0

&lt;210&gt; 1

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&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(2382)

&lt;400&gt; 1

|                     |                 |                             |    |
|---------------------|-----------------|-----------------------------|----|
| atg tgg atc cag gtt | cg acc atg gat  | ggg agg cag acc cac acg gtg | 48 |
| Met Trp Ile Gln Val | Arg Thr Met Asp | Gly Arg Gln Thr His Thr Val |    |
| 1                   | 5               | 10                          | 15 |

|                     |                     |                             |    |
|---------------------|---------------------|-----------------------------|----|
| gac tcg ctg tcc agg | ctg acc aag gtg gag | ctg agg cgg aag atc         | 96 |
| Asp Ser Leu Ser Arg | Leu Thr Lys Val     | Glu Glu Leu Arg Arg Lys Ile |    |
| 20                  | 25                  | 30                          |    |

|                     |                             |                     |     |
|---------------------|-----------------------------|---------------------|-----|
| cag gag ctg ttc cac | gtg gag cca ggc ctg cag     | agg ctg ttc tac agg | 144 |
| Gln Glu Leu Phe His | Val Glu Pro Gly Leu Gln Arg | Leu Phe Tyr Arg     |     |
| 35                  | 40                          | 45                  |     |

|                     |                         |                     |     |
|---------------------|-------------------------|---------------------|-----|
| ggc aaa cag atg gag | gac ggc cat acc ctc ttc | gac tac gag gtc cgc | 192 |
| Gly Lys Gln Met Glu | Asp Gly His Thr Leu Phe | Asp Tyr Glu Val Arg |     |
| 50                  | 55                      | 60                  |     |

|                     |                     |                         |     |
|---------------------|---------------------|-------------------------|-----|
| ctg aat gac acc atc | cag ctc ctg gtc cgc | cag agc ctc gtg ctc ccc | 240 |
| Leu Asn Asp Thr Ile | Gln Leu Leu Val Arg | Gln Ser Leu Val Leu Pro |     |
| 65                  | 70                  | 75                      | 80  |

|                     |                         |                     |     |
|---------------------|-------------------------|---------------------|-----|
| cac agc acc aag gag | cg gac tcc gag ctc tcc  | gac acc gac tcc ggc | 288 |
| His Ser Thr Lys Glu | Arg Asp Ser Glu Leu Ser | Asp Thr Asp Ser Gly |     |
| 85                  | 90                      | 95                  |     |

|                     |                     |                         |     |
|---------------------|---------------------|-------------------------|-----|
| tgc tgc ctg ggc cag | agt gag tca gac aag | tcc acc cac ggt gag     | 336 |
| Cys Cys Leu Gly Gln | Ser Glu Ser Asp Lys | Ser Ser Thr His Gly Glu |     |
| 100                 | 105                 | 110                     |     |

|                     |                         |                         |     |
|---------------------|-------------------------|-------------------------|-----|
| gcg gcc gcc gag act | gac agc agg cca gcc     | gat gag gac atg tgg gat | 384 |
| Ala Ala Ala Glu Thr | Asp Ser Arg Pro Ala Asp | Glu Asp Met Trp Asp     |     |
| 115                 | 120                     | 125                     |     |

|                     |                     |                         |     |
|---------------------|---------------------|-------------------------|-----|
| gag acg gaa ttg ggg | ctg tac aag gtc aat | gag tac gtc gat gct cgg | 432 |
| Glu Thr Glu Leu Gly | Leu Tyr Lys Val Asn | Glu Tyr Val Asp Ala Arg |     |
| 130                 | 135                 | 140                     |     |

|                     |                     |                         |     |
|---------------------|---------------------|-------------------------|-----|
| gac acg aac atg ggg | gcg tgg ttt gag gcg | cag gtg gtc agg gtg acg | 480 |
| Asp Thr Asn Met Gly | Ala Trp Phe Glu Ala | Gln Val Val Arg Val Thr |     |

| 145   | 150 | 155 | 160  |     |
|---|-----|-----|------|-----|
| cg <sup>g</sup> a <sup>a</sup> g <sup>c</sup> c <sup>c</sup> t <sup>c</sup> c <sup>g</sup> g <sup>a</sup> g <sup>a</sup> c <sup>c</sup> t <sup>g</sup> c <sup>a</sup> g <sup>t</sup> c <sup>c</sup> a <sup>g</sup> g <sup>c</sup> c <sup>g</sup><br>Arg Lys Ala Pro Ser Arg Asp Glu Pro Cys Ser Ser Thr Ser Arg Pro   |     |     |      | 528 |
| 165   | 170 | 175 |      |     |
| g <sup>c</sup> g <sup>t</sup> g <sup>g</sup> g <sup>a</sup> g <sup>a</sup> c <sup>t</sup> a <sup>t</sup> t <sup>a</sup> c <sup>c</sup> g <sup>t</sup> a <sup>a</sup> a <sup>a</sup> t <sup>a</sup> c <sup>g</sup> g <sup>a</sup> c <sup>a</sup> t <sup>a</sup> c <sup>c</sup> g <sup>c</sup><br>Ala Leu Glu Glu Asp Val Ile Tyr His Val Lys Tyr Asp Asp Tyr Pro |     |     | 576  |     |
| 180   | 185 | 190 |      |     |
| g <sup>a</sup> a <sup>a</sup> g <sup>g</sup> g <sup>t</sup> g <sup>t</sup> c <sup>a</sup> g <sup>a</sup> t <sup>a</sup> g <sup>a</sup> t <sup>c</sup> a <sup>g</sup> g <sup>a</sup> c <sup>t</sup> c <sup>g</sup> a <sup>g</sup> c <sup>g</sup> g <sup>c</sup> g <sup>c</sup><br>Glu Asn Gly Val Val Gln Met Asn Ser Arg Asp Val Arg Ala Arg Ala                |     |     | 624  |     |
| 195   | 200 | 205 |      |     |
| c <sup>g</sup> c <sup>c</sup> a <sup>c</sup> a <sup>t</sup> a <sup>t</sup> a <sup>a</sup> g <sup>t</sup> g <sup>a</sup> c <sup>a</sup> g <sup>t</sup> g <sup>g</sup> c <sup>a</sup> g <sup>t</sup> g <sup>t</sup> g <sup>c</sup> a <sup>t</sup> g <sup>t</sup><br>Arg Thr Ile Ile Lys Trp Gln Asp Leu Glu Val Gly Gln Val Val Met                               |     |     | 672  |     |
| 210   | 215 | 220 |      |     |
| c <sup>t</sup> c <sup>a</sup> a <sup>a</sup> t <sup>a</sup> a <sup>a</sup> c <sup>c</sup> g <sup>a</sup> a <sup>a</sup> c <sup>c</sup> a <sup>a</sup> g <sup>g</sup> c <sup>g</sup> g <sup>g</sup> c <sup>t</sup> t <sup>c</sup> t <sup>g</sup> t <sup>a</sup> c <sup>a</sup><br>Leu Asn Tyr Asn Pro Asp Asn Pro Lys Glu Arg Gly Phe Trp Tyr Asp                |     |     | 720  |     |
| 225   | 230 | 235 | 240  |     |
| g <sup>c</sup> g <sup>a</sup> a <sup>t</sup> c <sup>c</sup> a <sup>g</sup> a <sup>a</sup> c <sup>g</sup> g <sup>a</sup> a <sup>c</sup> g <sup>a</sup> c <sup>g</sup> g <sup>c</sup> g <sup>a</sup> a <sup>a</sup> c <sup>t</sup> t <sup>a</sup><br>Ala Glu Ile Ser Arg Lys Arg Glu Thr Arg Thr Ala Arg Glu Leu Tyr  |     |     | 768  |     |
| 245   | 250 | 255 |      |     |
| g <sup>c</sup> c <sup>a</sup> a <sup>a</sup> g <sup>t</sup> g <sup>t</sup> c <sup>t</sup> g <sup>g</sup> g <sup>a</sup> t <sup>t</sup> g <sup>a</sup> t <sup>c</sup> t <sup>g</sup> a <sup>a</sup> g <sup>c</sup> t <sup>t</sup> c <sup>g</sup> a <sup>t</sup> a <sup>t</sup><br>Ala Asn Val Val Leu Gly Asp Asp Ser Leu Asn Asp Cys Arg Ile Ile                |     |     | 816  |     |
| 260   | 265 | 270 |      |     |
| t <sup>t</sup> c <sup>t</sup> g <sup>g</sup> a <sup>a</sup> g <sup>t</sup> t <sup>c</sup> a <sup>a</sup> g <sup>t</sup> t <sup>t</sup> g <sup>a</sup> c <sup>g</sup> c <sup>g</sup> g <sup>g</sup> t <sup>t</sup> g <sup>a</sup> g <sup>g</sup> a <sup>c</sup> c <sup>c</sup><br>Phe Val Asp Glu Val Phe Lys Ile Glu Arg Pro Gly Glu Gly Ser Pro                |     |     | 864  |     |
| 275   | 280 | 285 |      |     |
| a <sup>t</sup> g <sup>t</sup> g <sup>t</sup> a <sup>a</sup> c <sup>c</sup> a <sup>t</sup> g <sup>a</sup> c <sup>g</sup> a <sup>a</sup> g <sup>c</sup> a <sup>g</sup> c <sup>g</sup> g <sup>g</sup> c <sup>c</sup> t <sup>c</sup> t <sup>g</sup> a <sup>a</sup> c <sup>a</sup><br>Met Val Asp Asn Pro Met Arg Arg Lys Ser Gly Pro Ser Cys Lys His                |     |     | 912  |     |
| 290   | 295 | 300 |      |     |
| t <sup>c</sup> g <sup>a</sup> a <sup>a</sup> g <sup>c</sup> g <sup>t</sup> a <sup>a</sup> c <sup>a</sup> g <sup>a</sup> c <sup>t</sup> t <sup>c</sup> a <sup>g</sup> g <sup>t</sup> c <sup>t</sup> g <sup>c</sup> g <sup>c</sup> t <sup>c</sup> a <sup>c</sup><br>Cys Lys Asp Asp Val Asn Arg Leu Cys Arg Val Cys Ala Cys His Leu                               |     |     | 960  |     |
| 305   | 310 | 315 | 320  |     |
| t <sup>c</sup> g <sup>g</sup> g <sup>g</sup> c <sup>g</sup> c <sup>a</sup> g <sup>c</sup> c <sup>c</sup> g <sup>a</sup> a <sup>a</sup> c <sup>t</sup> a <sup>t</sup> t <sup>c</sup> g <sup>a</sup> t <sup>t</sup> g <sup>a</sup> t <sup>c</sup><br>Cys Gly Arg Gln Asp Pro Asp Lys Gln Leu Met Cys Asp Glu Cys  |     |     | 1008 |     |
| 325   | 330 | 335 |      |     |
| g <sup>a</sup> c <sup>a</sup> t <sup>g</sup> g <sup>c</sup> t <sup>t</sup> c <sup>a</sup> t <sup>c</sup> t <sup>a</sup> t <sup>c</sup> g <sup>c</sup> c <sup>g</sup> c <sup>c</sup> c <sup>t</sup> a <sup>g</sup> t <sup>g</sup> t <sup>t</sup> t <sup>a</sup><br>Asp Met Ala Phe His Ile Tyr Cys Leu Asp Pro Pro Leu Ser Ser Val                               |     |     | 1056 |     |
| 340   | 345 | 350 |      |     |
| c <sup>c</sup> a <sup>g</sup> g <sup>a</sup> g <sup>c</sup> g <sup>a</sup> t <sup>g</sup> t <sup>a</sup> t <sup>c</sup> g <sup>c</sup> t <sup>c</sup> g <sup>a</sup> a <sup>a</sup> t <sup>g</sup> g <sup>c</sup> a <sup>g</sup> c <sup>a</sup><br>Pro Ser Glu Asp Glu Trp Tyr Cys Pro Glu Cys Arg Asn Asp Ala Ser  |     |     | 1104 |     |
| 355   | 360 | 365 |      |     |
| g <sup>a</sup> g <sup>t</sup> g <sup>t</sup> a <sup>c</sup> t <sup>g</sup> g <sup>c</sup> g <sup>g</sup> a <sup>a</sup> g <sup>c</sup> c <sup>t</sup> a <sup>g</sup> a <sup>a</sup> g <sup>a</sup> a <sup>a</sup> t <sup>t</sup> g <sup>c</sup><br>Glu Val Val Leu Ala Gly Glu Arg Leu Arg Glu Ser Lys Lys Asn Ala  |     |     | 1152 |     |
| 370   | 375 | 380 |      |     |
| a <sup>a</sup> g <sup>t</sup> g <sup>c</sup> t <sup>c</sup> g <sup>c</sup> a <sup>c</sup> t <sup>g</sup> t <sup>c</sup> t <sup>a</sup> c <sup>a</sup> g <sup>c</sup> g <sup>g</sup> g <sup>a</sup> a <sup>a</sup> g <sup>g</sup> g <sup>c</sup><br>Lys Met Ala Ser Ala Thr Ser Ser Gln Arg Asp Trp Gly Lys Gly  |     |     | 1200 |     |
| 385   | 390 | 395 | 400  |     |

|   |      |
|---|------|
| atg gcc tgt gtg ggc cgc acc aag gaa tgt acc atc gtc ccg tcc aac<br>Met Ala Cys Val Gly Arg Thr Lys Glu Cys Thr Ile Val Pro Ser Asn<br>405 410 415     | 1248 |
| cac tac gga ccc atc ccg ggg atc ccc gtg ggc acc atg tgg cggtt<br>His Tyr Gly Pro Ile Pro Gly Ile Pro Val Gly Thr Met Trp Arg Phe<br>420 425 430       | 1296 |
| cga gtc cag gtc agc gag tcg ggt gtc cat cgg ccc cac gtg gct ggc<br>Arg Val Gln Val Ser Glu Ser Gly Val His Arg Pro His Val Ala Gly<br>435 440 445     | 1344 |
| atc cat ggc cgg agc aac gac gga tcg tac tcc cta gtc ctg gcg ggg<br>Ile His Gly Arg Ser Asn Asp Gly Ser Tyr Ser Leu Val Leu Ala Gly<br>450 455 460     | 1392 |
| ggc tat gag gat gat gtg gac cat ggg aat ttt ttc aca tac acg ggt<br>Gly Tyr Glu Asp Asp Val Asp His Gly Asn Phe Phe Thr Tyr Thr Gly<br>465 470 475 480 | 1440 |
| agt ggt ggt cga gat ctt tcc ggc aac aag agg acc gcg gaa cag tct<br>Ser Gly Gly Arg Asp Leu Ser Gly Asn Lys Arg Thr Ala Glu Gln Ser<br>485 490 495     | 1488 |
| tgt gat cag aaa ctc acc aac acc agg gcg ctg gct ctc aac tgc<br>Cys Asp Gln Lys Leu Thr Asn Thr Asn Arg Ala Leu Ala Leu Asn Cys<br>500 505 510         | 1536 |
| ttt gct ccc atc aat gac caa gaa ggg gcc gag gcc aag gac tgg cggtt<br>Phe Ala Pro Ile Asn Asp Gln Glu Gly Ala Glu Ala Lys Asp Trp Arg<br>515 520 525   | 1584 |
| tcg ggg aag ccg gtc agg gtg gtg cgc aat gtc aag ggt ggc aag aat<br>Ser Gly Lys Pro Val Arg Val Val Arg Asn Val Lys Gly Gly Lys Asn<br>530 535 540     | 1632 |
| agc aag tac gcc ccc gct gag ggc aac cgc tac gat ggc atc tac aag<br>Ser Lys Tyr Ala Pro Ala Glu Gly Asn Arg Tyr Asp Gly Ile Tyr Lys<br>545 550 555 560 | 1680 |
| gtt gtg aaa tac tgg ccc gag aag ggg aag tcc ggg ttt ctc gtg tgg<br>Val Val Lys Tyr Trp Pro Glu Lys Gly Lys Ser Gly Phe Leu Val Trp<br>565 570 575     | 1728 |
| cgc tac ctt ctg cgg agg gac gat gat gag cct ggc cct tgg acg aag<br>Arg Tyr Leu Leu Arg Arg Asp Asp Glu Pro Gly Pro Trp Thr Lys<br>580 585 590         | 1776 |
| gag ggg aag gac cgg atc aag aag ctg ggg ctg acc atg cag tat cca<br>Glu Gly Lys Asp Arg Ile Lys Lys Leu Gly Leu Thr Met Gln Tyr Pro<br>595 600 605     | 1824 |
| gaa ggc tac ctg gaa gcc ctg gcc aac cga gag cga gag aag gag aac<br>Glu Gly Tyr Leu Glu Ala Leu Ala Asn Arg Glu Arg Glu Lys Glu Asn<br>610 615 620     | 1872 |
| agc aag agg gag gag cag cag gag ggg ggc ttc gcg tcc ccc<br>Ser Lys Arg Glu Glu Glu Gln Gln Glu Gly Gly Phe Ala Ser Pro<br>625 630 635 640             | 1920 |
| agg acg ggc aag ggc aag tgg aag cgg aag tcg gca gga ggt ggc ccg.<br>Arg Thr Gly Lys Gly Lys Trp Lys Arg Lys Ser Ala Gly Gly Pro                       | 1968 |

| 645   | 650 | 655 |      |
|---|-----|-----|------|
| agc agg gcc ggg tcc ccg cgc cgg aca tcc aag aaa acc aag gtg gag<br>Ser Arg Ala Gly Ser Pro Arg Arg Thr Ser Lys Lys Thr Lys Val Glu<br>660             | 665 | 670 | 2016 |
| ccc tac agt ctc acg gcc cag cag agc agc ctc atc aga gag gac aag<br>Pro Tyr Ser Leu Thr Ala Gln Gln Ser Ser Leu Ile Arg Glu Asp Lys<br>675             | 680 | 685 | 2064 |
| agc aac gcc aag ctg tgg aat gag gtc ctg gcg tca ctc aag gac cgg<br>Ser Asn Ala Lys Leu Trp Asn Glu Val Leu Ala Ser Leu Lys Asp Arg<br>690             | 695 | 700 | 2112 |
| ccg gcg agc ggc agc ccg ttc cag ttg ttc ctg agt aaa gtg gag gag<br>Pro Ala Ser Gly Ser Pro Phe Gln Leu Phe Leu Ser Lys Val Glu Glu<br>705             | 710 | 715 | 2160 |
| acg ttc cag tgt atc tgc tgt cag gag ctg gtg ttc cgg ccc atc acg<br>Thr Phe Gln Cys Ile Cys Cys Gln Glu Leu Val Phe Arg Pro Ile Thr<br>725             | 730 | 735 | 2208 |
| acc gtg tgc cag cac aac gtg tgc aag gac tgc ctg gac aga tcc ttt<br>Thr Val Cys Gln His Asn Val Cys Lys Asp Cys Leu Asp Arg Ser Phe<br>740             | 745 | 750 | 2256 |
| cg <sup>g</sup> gca cag gtg ttc agc tgc cct gcc tgc cgc tac gac ctg ggc cgc<br>Arg Ala Gln Val Phe Ser Cys Pro Ala Cys Arg Tyr Asp Leu Gly Arg<br>755 | 760 | 765 | 2304 |
| agc tat gcc atg cag gtg aac cag cct ctg cag acc gtc ctc aac cag<br>Ser Tyr Ala Met Gln Val Asn Gln Pro Leu Gln Thr Val Leu Asn Gln<br>770             | 775 | 780 | 2352 |
| ctc ttc ccc ggc tac ggc aat ggc cgg tga<br>Leu Phe Pro Gly Tyr Gly Asn Gly Arg<br>785   | 790 |     | 2382 |
| <210> 2   |     |     |      |
| <211> 793   |     |     |      |
| <212> PRT   |     |     |      |
| <213> Homo sapiens  |     |     |      |
| <400> 2   |     |     |      |
| Met Trp Ile Gln Val Arg Thr Met Asp Gly Arg Gln Thr His Thr Val<br>1 5 10 15  |     |     |      |
| Asp Ser Leu Ser Arg Leu Thr Lys Val Glu Glu Leu Arg Arg Lys Ile<br>20 25 30   |     |     |      |
| Gln Glu Leu Phe His Val Glu Pro Gly Leu Gln Arg Leu Phe Tyr Arg<br>35 40 45   |     |     |      |
| Gly Lys Gln Met Glu Asp Gly His Thr Leu Phe Asp Tyr Glu Val Arg<br>50 55 60   |     |     |      |
| Leu Asn Asp Thr Ile Gln Leu Leu Val Arg Gln Ser Leu Val Leu Pro<br>65 70 75 80  |     |     |      |
| His Ser Thr Lys Glu Arg Asp Ser Glu Leu Ser Asp Thr Asp Ser Gly<br>85 90 95   |     |     |      |

Cys Cys Leu Gly Gln Ser Glu Ser Asp Lys Ser Ser Thr His Gly Glu  
 100 105 110  
 Ala Ala Ala Glu Thr Asp Ser Arg Pro Ala Asp Glu Asp Met Trp Asp  
 115 120 125  
 Glu Thr Glu Leu Gly Leu Tyr Lys Val Asn Glu Tyr Val Asp Ala Arg  
 130 135 140  
 Asp Thr Asn Met Gly Ala Trp Phe Glu Ala Gln Val Val Arg Val Thr  
 145 150 155 160  
 Arg Lys Ala Pro Ser Arg Asp Glu Pro Cys Ser Ser Thr Ser Arg Pro  
 165 170 175  
 Ala Leu Glu Glu Asp Val Ile Tyr His Val Lys Tyr Asp Asp Tyr Pro  
 180 185 190  
 Glu Asn Gly Val Val Gln Met Asn Ser Arg Asp Val Arg Ala Arg Ala  
 195 200 205  
 Arg Thr Ile Ile Lys Trp Gln Asp Leu Glu Val Gly Gln Val Val Met  
 210 215 220  
 Leu Asn Tyr Asn Pro Asp Asn Pro Lys Glu Arg Gly Phe Trp Tyr Asp  
 225 230 235 240  
 Ala Glu Ile Ser Arg Lys Arg Glu Thr Arg Thr Ala Arg Glu Leu Tyr  
 245 250 255  
 Ala Asn Val Val Leu Gly Asp Asp Ser Leu Asn Asp Cys Arg Ile Ile  
 260 265 270  
 Phe Val Asp Glu Val Phe Lys Ile Glu Arg Pro Gly Glu Gly Ser Pro  
 275 280 285  
 Met Val Asp Asn Pro Met Arg Arg Lys Ser Gly Pro Ser Cys Lys His  
 290 295 300  
 Cys Lys Asp Asp Val Asn Arg Leu Cys Arg Val Cys Ala Cys His Leu  
 305 310 315 320  
 Cys Gly Gly Arg Gln Asp Pro Asp Lys Gln Leu Met Cys Asp Glu Cys  
 325 330 335  
 Asp Met Ala Phe His Ile Tyr Cys Leu Asp Pro Pro Leu Ser Ser Val  
 340 345 350  
 Pro Ser Glu Asp Glu Trp Tyr Cys Pro Glu Cys Arg Asn Asp Ala Ser  
 355 360 365  
 Glu Val Val Leu Ala Gly Glu Arg Leu Arg Glu Ser Lys Lys Asn Ala  
 370 375 380  
 Lys Met Ala Ser Ala Thr Ser Ser Ser Gln Arg Asp Trp Gly Lys Gly  
 385 390 395 400  
 Met Ala Cys Val Gly Arg Thr Lys Glu Cys Thr Ile Val Pro Ser Asn  
 405 410 415  
 His Tyr Gly Pro Ile Pro Gly Ile Pro Val Gly Thr Met Trp Arg Phe

|   |     |     |
|---|-----|-----|
| 420   | 425 | 430 |
| Arg Val Gln Val Ser Glu Ser Gly Val His Arg Pro His Val Ala Gly |     |     |
| 435   | 440 | 445 |
| Ile His Gly Arg Ser Asn Asp Gly Ser Tyr Ser Leu Val Leu Ala Gly |     |     |
| 450   | 455 | 460 |
| Gly Tyr Glu Asp Asp Val Asp His Gly Asn Phe Phe Thr Tyr Thr Gly |     |     |
| 465   | 470 | 475 |
| Ser Gly Gly Arg Asp Leu Ser Gly Asn Lys Arg Thr Ala Glu Gln Ser |     |     |
| 485   | 490 | 495 |
| Cys Asp Gln Lys Leu Thr Asn Thr Asn Arg Ala Leu Ala Leu Asn Cys |     |     |
| 500   | 505 | 510 |
| Phe Ala Pro Ile Asn Asp Gln Glu Gly Ala Glu Ala Lys Asp Trp Arg |     |     |
| 515   | 520 | 525 |
| Ser Gly Lys Pro Val Arg Val Val Arg Asn Val Lys Gly Gly Lys Asn |     |     |
| 530   | 535 | 540 |
| Ser Lys Tyr Ala Pro Ala Glu Gly Asn Arg Tyr Asp Gly Ile Tyr Lys |     |     |
| 545   | 550 | 555 |
| Val Val Lys Tyr Trp Pro Glu Lys Gly Lys Ser Gly Phe Leu Val Trp |     |     |
| 565   | 570 | 575 |
| Arg Tyr Leu Leu Arg Arg Asp Asp Asp Glu Pro Gly Pro Trp Thr Lys |     |     |
| 580   | 585 | 590 |
| Glu Gly Lys Asp Arg Ile Lys Lys Leu Gly Leu Thr Met Gln Tyr Pro |     |     |
| 595   | 600 | 605 |
| Glu Gly Tyr Leu Glu Ala Leu Ala Asn Arg Glu Arg Glu Lys Glu Asn |     |     |
| 610   | 615 | 620 |
| Ser Lys Arg Glu Glu Glu Glu Gln Gln Glu Gly Gly Phe Ala Ser Pro |     |     |
| 625   | 630 | 635 |
| Arg Thr Gly Lys Gly Lys Trp Lys Arg Lys Ser Ala Gly Gly Pro     |     |     |
| 645   | 650 | 655 |
| Ser Arg Ala Gly Ser Pro Arg Arg Thr Ser Lys Lys Thr Lys Val Glu |     |     |
| 660   | 665 | 670 |
| Pro Tyr Ser Leu Thr Ala Gln Gln Ser Ser Leu Ile Arg Glu Asp Lys |     |     |
| 675   | 680 | 685 |
| Ser Asn Ala Lys Leu Trp Asn Glu Val Leu Ala Ser Leu Lys Asp Arg |     |     |
| 690   | 695 | 700 |
| Pro Ala Ser Gly Ser Pro Phe Gln Leu Phe Leu Ser Lys Val Glu Glu |     |     |
| 705   | 710 | 715 |
| Thr Phe Gln Cys Ile Cys Cys Gln Glu Leu Val Phe Arg Pro Ile Thr |     |     |
| 725   | 730 | 735 |
| Thr Val Cys Gln His Asn Val Cys Lys Asp Cys Leu Asp Arg Ser Phe |     |     |
| 740   | 745 | 750 |

Arg Ala Gln Val Phe Ser Cys Pro Ala Cys Arg Tyr Asp Leu Gly Arg  
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Ser Tyr Ala Met Gln Val Asn Gln Pro Leu Gln Thr Val Leu Asn Gln  
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Leu Phe Pro Gly Tyr Gly Asn Gly Arg  
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tgc aag gac gac gtg aac aga ctc tgc agc 78  
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Cys Lys Asp Asp Val Asn Arg Leu Cys Ser  
20 25

<210> 7  
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<222> (1)..(525)

|         |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| <400> 7 |     |     |     |     |     |     |     |     |     | 48  |     |     |     |     |     |     |
| cga     | gag | aag | gag | aac | agc | aag | agg | gag | gag | gag | cag | cag | gag | ggg |     |     |
| Arg     | Glu | Lys | Glu | Asn | Ser | Lys | Arg | Glu | Glu | Glu | Gln | Gln | Glu | Gly |     |     |
| 1       |     |     | 5   |     |     |     |     | 10  |     |     |     | 15  |     |     |     |     |
| ggc     | tcc | gcg | tcc | ccc | agg | acg | ggc | aag | ggc | aag | tgg | aag | cgg | aag | tgc |     |
| Gly     | Phe | Ala | Ser | Pro | Arg | Thr | Gly | Lys | Gly | Lys | Trp | Lys | Arg | Lys | Ser |     |
|         |     |     | 20  |     |     |     |     | 25  |     |     |     | 30  |     |     | 96  |     |
| gca     | gga | ggt | ggc | ccg | agc | agg | gcc | ggg | tcc | ccg | cgc | cgg | aca | tcc | aag |     |
| Ala     | Gly | Gly | Gly | Pro | Ser | Arg | Ala | Gly | Ser | Pro | Arg | Arg | Thr | Ser | Lys |     |
|         |     |     | 35  |     |     |     |     | 40  |     |     |     | 45  |     |     | 14  |     |
| aaa     | acc | aag | gtg | gag | ccc | tac | agt | ctc | acg | gcc | cag | cag | agc | agc | ctc |     |
| Lys     | Thr | Lys | Val | Glu | Pro | Tyr | Ser | Leu | Thr | Ala | Gln | Gln | Ser | Ser | Leu |     |
|         |     |     | 50  |     |     |     |     | 55  |     |     |     | 60  |     |     | 192 |     |
| atc     | aga | gag | gac | aag | agc | aac | gcc | aag | ctg | tgg | aat | gag | gtc | ctg | gcg |     |
| Ile     | Arg | Glu | Asp | Lys | Ser | Asn | Ala | Lys | Leu | Trp | Asn | Glu | Val | Leu | Ala |     |
|         |     |     | 65  |     |     |     |     | 70  |     |     | 75  |     |     |     | 240 |     |
| tca     | ctc | aag | gac | cg  | ccg | g   | gc  | agc | ggc | ccg | t   | tc  | cag | ttg | ttc | ctg |
| Ser     | Leu | Lys | Asp | Arg | Pro | Ala | Ser | Gly | Ser | Pro | Phe | Gln | Leu | Phe | Leu |     |
|         |     |     | 85  |     |     |     |     | 90  |     |     |     | 95  |     |     | 288 |     |
| agt     | aaa | gtg | gag | gag | acg | ttc | cag | tgt | atc | tgc | tgt | cag | gag | ctg | gtg |     |
| Ser     | Lys | Val | Glu | Glu | Thr | Phe | Gln | Cys | Ile | Cys | Cys | Gln | Glu | Leu | Val |     |
|         |     |     | 100 |     |     |     |     | 105 |     |     |     | 110 |     |     | 336 |     |
| ttc     | cg  | cc  | atc | ac  | gt  | tg  | cag | cac | aa  | gt  | tg  | aag | gac | tg  |     |     |
| he      | Arg | Pro | Ile | Thr | Thr | Val | Cys | Gln | His | Asn | Val | Cys | Lys | Asp | Cys |     |
|         |     |     | 115 |     |     |     |     | 120 |     |     |     | 125 |     |     | 384 |     |
| ttg     | gac | aga | tcc | ttt | cg  | gca | cag | gt  | ttc | agc | tgc | cct | gcc | tgc | cg  |     |
| eu      | Asp | Arg | Ser | Phe | Arg | Ala | Gln | Val | Phe | Ser | Cys | Pro | Ala | Cys | Arg |     |
|         |     |     | 130 |     |     |     |     | 135 |     |     |     | 140 |     |     | 432 |     |
| ac      | gac | ctg | gg  | cg  | agc | ta  | g   | cc  | at  | g   | tg  | aa  | c   | c   |     |     |
| yr      | Asp | Leu | Gly | Arg | Ser | Tyr | Ala | Met | Gln | Val | Asn | Gln | Pro | Leu | Gln |     |
|         |     |     | 45  |     |     |     |     | 150 |     |     | 155 |     |     | 160 |     |     |
| cc      | gtc | ctc | aa  | c   | tg  | ct  | tt  | cc  | gg  | ta  | at  | gg  | cg  | tg  |     |     |
| hr      | Val | Leu | Asn | Gln | Leu | Phe | Pro | Gly | Tyr | Gly | Asn | Gly | Arg |     | 525 |     |
|         |     |     | 165 |     |     |     |     | 170 |     |     |     | 175 |     |     |     |     |

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<212> PRT  
<213> Homo sapiens

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20 25 30  
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35 40 45  
Lys Thr Lys Val Glu Pro Tyr Ser Leu Thr Ala Gln Gln Ser Ser Leu  
50 55 60  
Ile Arg Glu Asp Lys Ser Asn Ala Lys Leu Trp Asn Glu Val Leu Ala  
65 70 75 80  
Ser Leu Lys Asp Arg Pro Ala Ser Gly Ser Pro Phe Gln Leu Phe Leu  
85 90 95  
Ser Lys Val Glu Glu Thr Phe Gln Cys Ile Cys Cys Gln Glu Leu Val  
100 105 110  
Phe Arg Pro Ile Thr Thr Val Cys Gln His Asn Val Cys Lys Asp Cys  
115 120 125  
Leu Asp Arg Ser Phe Arg Ala Gln Val Phe Ser Cys Pro Ala Cys Arg  
130 135 140  
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ggcctgcaga ggctgttcta caggggcaaa cagatggagg acggccatac cctcttcgac 180  
tacgagggtcc gcctgaatga caccatccag ctcctggtcc gccagagcct cgtgctcccc 240  
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<210> 10  
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 ccctcccccggg acgagccctg cagctccacg tccaggccgg cgctggagga ggacgtcatt 180  
 taccacgtga aatacgacga ctacccggag aacggcgtgg tccagatgaa ctccaggggac 240  
 gtccgagcgc gcgcccccac catcatcaag tggcaggacc tggaggtggg ccaggtggtc 300  
 atgctaact acaaaccggc caaccccaag gagcggggct tctgttacga cgcggagatc 360  
 tccaggaagc gcgagaccag gacggcgcgg gaactctacg ccaacgtggt gctggggat 420  
 gattctctga acgactgtcg gatcatcttc gtggacgaag tcttcaagat tgagcggccg 480  
 ggtgaaggga gcccc 495

&lt;210&gt; 11

&lt;211&gt; 915

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 11

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 gaggacgagt ggtactgccc tggatgcgg aatgatgcca gcgagggtgt actggcggga 180  
 gagcggctga gagagagcaa gaagaatgcg aagatggct cggccacatc gtcctcacag 240  
 cgggactggg gcaaggccat ggcctgtgtg ggcgcacca aggaatgtac catcgcccg 300  
 tccaaaccact acggacccat cccgggatc cccgtggca ccatgtggcg gttccgagtc 360  
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 gacggatcgt actcccttagt cctggcgaaa ggctatgagg atgatgtgaa ccatggaaat 480  
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 gtgtggcgct accttctgcg gagggacgat gatgacccctg gcccttggac gaaggagggg 840  
 aaggaccggg tcaagaagct gggctgacc atgcgtatc cagaaggcta cctggaaagcc 900  
 ctggccaacc gagag 915

LEGENDS TO FIGURESFIG. 2

Proteine endogène

Endogenous protein

5

FIG. 5

//row A//

cerveau entier

entire brain

amygdale

tonsil

10 noyau caudé

caudate nucleus

cervelet

cerebellum

cortex cérébral

cerebral cortex

lobe frontal

frontal lobe

hippocampe

hippocampus

15 medulla oblongata

medulla oblongata

//row B//

lobe occipital

occipital lobe

putamen

putamen

20 substancia nigra

substantia nigra

lobe temporal

temporal lobe

thalamus

thalamus

noyau subthalamique

subthalamic nucleus

moelle épinière

spinal marrow

25

//row C//

coeur

heart

aorte

aorta

muscle squelettique

skeletal muscle

30 colon

colon

vessie

bladder

utérus

uterus

prostate

prostate

|    |                        |                      |
|----|------------------------|----------------------|
|    | estomac                | stomach              |
|    | //row D//              |                      |
|    | testicule              | testicle             |
| 5  | ovaire                 | ovary                |
|    | pancréas               | pancreas             |
|    | glande pituitaire      | pituitary gland      |
|    | glande surrénale       | adrenal gland        |
|    | glande thyroïde        | thyroid gland        |
| 10 | glande salivaire       | salivary gland       |
|    | gland mammaire         | mammary gland        |
|    | //row E//              |                      |
|    | rein                   | kidney               |
| 15 | foie                   | liver                |
|    | intestin grèle         | small intestine      |
|    | rate                   | spleen               |
|    | thymus                 | thymus               |
|    | leucocyte périphérique | peripheral leukocyte |
| 20 | ganglion lymphatique   | lymph gland          |
|    | moelle osseuse         | bone marrow          |
|    | //row F//              |                      |
|    | appendice              | appendix             |
| 25 | poumon                 | lung                 |
|    | trachée artère         | trachea artery       |
|    | placenta               | placenta             |
|    | //row G//              |                      |
| 30 | cerveau foetal         | foetal brain         |
|    | coeur foetal           | foetal heart         |
|    | rein foetal            | foetal kidney        |
|    | foie foetal            | foetal liver         |

|    |                            |                     |
|----|----------------------------|---------------------|
|    | rate foetale               | foetal spleen       |
|    | thymus foetal              | foetal thymus       |
|    | poumon foetal              | foetal lung         |
| 5  | //row H//                  |                     |
|    | ARN total de levure 100 ng | total yeast RNA 100 |
|    | ng                         |                     |
|    | ARN t de levure 100 ng     | t yeast RNA 100 ng  |
|    | ARNr E. coli 100 ng        | E. coli rRNA 100 ng |
| 10 | ADN E. coli 100 ng         | E. coli DNA 100 ng  |
|    | Poly c(A) 100 ng           | Poly c(A) 100 ng    |
|    | ADN ColI humain 100 ng     | Human ColI DNA 100  |
|    | ng                         |                     |
|    | ADN humain 100 ng          | Human DNA 100 ng    |
| 15 | ADN humain 500 ng          | Human DNA 500 ng    |

CLAIMS

1. Isolated polypeptide called ICBP90 (inverted CCAAT box binding protein 90) with amino acid sequence SEQ ID No. 2.

5 2. Isolated polypeptide characterized in that it comprises a polypeptide chosen from:

a) a polypeptide with sequence SEQ ID No. 2, SEQ ID No. 4, SEQ ID No. 6 or SEQ ID No. 8;

b) a polypeptide variant of polypeptide with sequences of amino acids defined in a);

10 c) a polypeptide homologous with the polypeptide defined in a) or b) and including at least 80% homology, preferably 90% with said polypeptide of a);

d) a fragment of at least 5 consecutive amino acids of a polypeptide defined in a), b) or c);

15 e) a biologically active fragment of a polypeptide defined in a), b) or c).

3. Polypeptide according to any one of Claims 1 to 2 and characterized in that it comprises of at least one domain for fixation to the DNA selected in the 20 group composed of a "zinc-finger" domain and a "leucine-zipper" domain.

4. Polypeptide according to Claim 3 characterized in that the DNA sequence on which said polypeptide is bound is a CCAAT box, preferably an inverted CCAAT box 25 (Inverted CCAAT box: ICB).

5. Isolated polynucleotide characterized in that it codes for a polypeptide according to Claim 1.

6. Polynucleotide according to Claim 5 with sequence SEQ ID No. 1.

7. Isolated polynucleotide characterized in that it comprises of a polynucleotide chosen from:

5 a) a polynucleotide with sequence SEQ ID No. 1, SEQ ID No. 3, SEQ ID No. 5 or SEQ ID No. 7 or in which the sequence is that of the RNA corresponding to the sequence SEQ No. 1, SEQ ID No. 3, SEQ ID No. 5 or SEQ ID No. 7;

10 b) a polynucleotide in which the sequence is complementary to the sequence of a polynucleotide defined in a),

c) a polynucleotide in which the sequence consists of at least 80% homology with a polynucleotide defined in a) or b),

15 d) a polynucleotide hybridizing under very stringent conditions with a polynucleotide sequence defined in a), b) or c),

20 e) a fragment of at least 15 consecutive nucleotides, preferably 21 consecutive nucleotides, and preferably 30 consecutive nucleotides of a nucleotide defined in a), b),  
c) or d) with the exception of the sequences SEQ ID No. 9, No. 10, No. 11 corresponding, respectively, to the human EST No. AI 083 773, AA 811 055, No. AA 488 755, N° AA 129 794 and No. AA 354 253.

25 8. Polynucleotide according to Claim 7 characterized in that it is labelled directly or indirectly with a radioactive compound or a nonradioactive compound.

30 9. Use of a polynucleotide according to Claim 8 as primer for amplification or polymerization of nucleic sequences.

10. Use of a polynucleotide according to Claim 8 as probe for detection of nucleic sequences.

11. Use of a polynucleotide according to Claim 8 as sense or antisense oligonucleotide to control the expression of the corresponding protein product.

12. Recombinant cloning vector of a polynucleotide  
5 according to one of Claims 5 to 8 and/or expression of a polypeptide according to one of Claims 1 to 4 characterized in that it contains a polynucleotide according to any one of Claims 5 to 8.

13. Vector according to Claim 12 characterized in  
10 that it consists of the parts enabling the expression possibly the secretion of said polypeptide in a host cell.

14. Host cell, characterized in that it is transformed by a vector according to one of Claims 12  
15 and 13.

15. Method for preparation of a recombinant polypeptide characterized in that a host cell is cultured according to Claim 14 under conditions enabling the expression and possibly the secretion of  
20 said recombinant polypeptide and that said recombinant polypeptide is recovered.

16. Recombinant polypeptide obtainable by a method according to Claim 15.

17. Monoclonal or polyclonal antibody and its  
25 fragments characterized in that it specifically binds a polypeptide according to one of Claims 1 to 4 and 16.

18. Monoclonal antibody according to Claim 18 specific for the human ICBP90 protein and capable of inhibiting the interaction between ICBP90 and the DNA  
30 sequence on which the protein ICBP90 is specifically bound.

19. Monoclonal antibody according to Claim 17 specific for the human ICBP90 protein and capable of inhibiting the interaction between ICBP90 and proteins with which ICBP90 interacts, said proteins preferably being ICBP90 itself or 5 proteins of a transcriptional complex.

20. Method for detection and/or measuring of a polypeptide according to one of Claims 1 to 4 and 16 in a biological sample, characterized in that it comprises the following steps:

10 a) putting the biological sample in contact with an antibody according to one of Claims 17 to 19;

b) revealing a formed antigen-antibody complex.

21. Kit for making use of a method according to Claim 20 in a biological sample by immunological reaction, 15 characterized in that it comprises the following parts:

a) a monoclonal or polyclonal antibody according to one of Claims 17 to 19;

b) if applicable, the reagents for the formation of the favourable medium for the immunological reaction;

20 c) the reagents enabling the detection of the antigen-antibody complex produced by the immunological reaction.

22. Method for detection and/or measurement of a polynucleotide according to any one of Claims 5 to 8 in biological sample, characterized in that it consists of the 25 following steps:

a) isolating the DNA from the biological sample to be analyzed, or obtaining cDNA from the RNA of the biological sample;

30 b) specific amplification of the DNA with the aid of primers according to Claim 9;

c) analysis of amplification products.

23. Kit for making use of a method according to Claim 22 in a biological sample characterized in that it comprises the following parts:

- a) a pair of nucleic primers according to Claim 9;
- 5 b) the reagents necessary for carrying out an amplification reaction of DNA;

c) possibly a component enabling the verification of the sequence of the amplified fragment, more particularly a probe according to Claim 10.

10 24. Method for detection and/or measurement of a nucleotide according to any one of Claims 5 to 8 in biological sample characterized in that it consists of the following steps:

- a) putting a probe according to Claim 10 in contact 15 with a biological sample;

b) detection and/or measurement of the hybrid formed between said probe and the DNA of the biological sample.

25. Kit for making use of a method according to Claim 24 in a biological sample characterized in that it comprises 20 the following parts:

- a) a probe according to Claim 10;
- b) the reagents necessary for using a hybridization reaction.

26. Method according to Claims 20, 22 and 24 for the 25 diagnosis of cellular proliferation.

27. Ligand screening method likely to affect the transcription activity of a gene the promoter of which consists of CCAAT and/or inverted CCAAT boxes (ICB) likely to bind a polypeptide according to Claims 1 to 4

and 16 and which consists of the following steps:

a) putting said polypeptide and one or more potential ligand(s) in the presence of reagents necessary for using a transcription reaction;

5 b) detection and/or measurement of the transcription activity.

28. Kit for making use of a method according to Claim 27 in a biological sample characterized in that it comprises the following parts:

10 a) a polypeptide according to Claims 1 to 4;

b) a ligand;

c) the reagents necessary for using a transcription reaction.

29. Compound according to one of Claims 1 to 8, 12  
15 to 14 and 16 to 19 as a drug.

30. Compound according to Claim 29 as a drug intended for the prevention and/or treatment of cancer.

31. Use of a compound according to Claims 29 and 20 for the preparation of a drug intended to modulate, increase or decrease cell proliferation.

32. Pharmaceutical composition for the preventive and curative treatment of cancer characterized in that it contains a therapeutically effective amount of a compound according to one of Claims 29 and 30 and a pharmaceutically acceptable vehicle.

33. Pharmaceutical composition characterized in that it comprises an antibody according to one of Claims 17 to 19 as screening agent conjugated with at least one agent selected from the group of 30 antiproliferative, antineoplastic or cytotoxic agents.

34. Product comprising at least one compound according to Claims 29 and 30 and at least another anticancer agent as combination product for simultaneous use, separate use or spread over time in

anticancer therapy.

35. Composition for the detection, localization and imagery of cancers, comprising an antibody according to any one of Claims 17 to 19, the antibody 5 is labelled directly or indirectly with a marker generating a signal selected from radioactive isotopes and nonisotope entities.

36. Method for the detection, localization and imagery of cancer, comprising the steps of:

10 a) parenteral injection of a composition according to Claim 35 in a human being;

b) accumulation after sufficient time of the labelled antibody at the cancer cells, then penetration of the labelled antibody within said cells, without 15 said antibody being bound substantially to the normal cells; and

c) detection of the signal by means of a signal detector; and

d) conversion of the detected signal into an image 20 of cancer cells.

6. Polynucleotide according to Claim 5 with sequence SEQ ID No. 1.

7. Isolated polynucleotide characterized in that it comprises of a polynucleotide chosen from:

5 a) a polynucleotide with sequence SEQ ID No. 1, SEQ ID No. 3, SEQ ID No. 5 or SEQ ID No. 7 or in which the sequence is that of the RNA corresponding to the sequence SEQ No. 1, SEQ ID No. 3, SEQ ID No. 5 or SEQ ID No. 7;

10 b) a polynucleotide in which the sequence is complementary to the sequence of a polynucleotide defined in a),

c) a polynucleotide in which the sequence consists of at least 80% homology with a polynucleotide defined in a) or b),

15 d) a polynucleotide hybridizing under very stringent conditions with a polynucleotide sequence defined in a), b) or c),

20 e) a fragment of at least 15 consecutive nucleotides, preferably 21 consecutive nucleotides, and preferably 30 consecutive nucleotides of a nucleotide defined in a), b),  
c) or d) with the exception of the sequences SEQ ID No. 9, No. 10, No. 11 corresponding, respectively, to the human EST No. AI 083 773, AA 811 055, No. AA 488 755, N° AA 129 794 and No. AA 354 253.

25 8. Polynucleotide according to Claim 7 characterized in that it is labelled directly or indirectly with a radioactive compound or a nonradioactive compound.

30 9. Use of a polynucleotide according to Claims 7 and 8 as primer for amplification or polymerization of nucleic sequences.

10. Use of a polynucleotide according to Claims 7 and 8 as probe for detection of nucleic sequences.

11. Use of a polynucleotide according to Claims 7 and 8 as sense or antisense oligonucleotide to control the expression of the corresponding protein product.

12. Recombinant cloning vector of a polynucleotide according to one of Claims 5 to 8 and/or expression of a polypeptide according to one of Claims 1 to 4 characterized in that it contains a polynucleotide according to any one of Claims 5 to 8.

13. Vector according to Claim 12 characterized in that it consists of the parts enabling the expression possibly the secretion of said polypeptide in a host cell.

14. Host cell, characterized in that it is transformed by a vector according to one of Claims 12 and 13.

15. Method for preparation of a recombinant polypeptide characterized in that a host cell is cultured according to Claim 14 under conditions enabling the expression and possibly the secretion of said recombinant polypeptide and that said recombinant polypeptide is recovered.

20 16. Recombinant polypeptide obtainable by a method according to Claim 15.

17. Monoclonal or polyclonal antibody and its fragments characterized in that it specifically binds a polypeptide according to one of Claims 1 to 4 and 16.

25 18. Monoclonal antibody according to Claim 18 specific for the human ICBP90 protein and capable of inhibiting the interaction between ICBP90 and the DNA sequence on which the protein ICBP90 is specifically bound.

19. Monoclonal antibody according to Claim 17 specific for the human ICBP90 protein and capable of inhibiting the interaction between ICBP90 and proteins with which ICBP90 interacts, said proteins preferably being ICBP90 itself or 5 proteins of a transcriptional complex.

20. Method for detection and/or measuring of a polypeptide according to one of Claims 1 to 4 and 16 in a biological sample, characterized in that it comprises the following steps:

10 a) putting the biological sample in contact with an antibody according to one of Claims 17 to 19;

b) revealing a formed antigen-antibody complex.

21. Kit for making use of a method according to Claim 20 in a biological sample by immunological reaction, 15 characterized in that it comprises the following parts:

a) a monoclonal or polyclonal antibody according to one of Claims 17 to 19;

b) if applicable, the reagents for the formation of the favourable medium for the immunological reaction;

20 c) the reagents enabling the detection of the antigen-antibody complex produced by the immunological reaction.

22. Method for detection and/or measurement of a polynucleotide according to any one of Claims 5 to 8 in biological sample, characterized in that it consists of the 25 following steps:

a) isolating the DNA from the biological sample to be analyzed, or obtaining cDNA from the RNA of the biological sample;

30 b) specific amplification of the DNA with the aid of polynucleotides according to Claim 7 or 8 used as primer;

c) analysis of amplification products.

23. Kit for making use of a method according to Claim 22 in a biological sample characterized in that it comprises the following parts:

5       a) a pair of polynucleotides according to Claim 7 or 8 used as primer;

      b) the reagents necessary for carrying out an amplification reaction of DNA;

10      c) possibly a component enabling the verification of the sequence of the amplified fragment, more particularly a polynucleotide according to Claim 7 or 8 used as probe.

24. Method for detection and/or measurement of a nucleotide according to any one of Claims 5 to 8 in biological sample characterized in that it consists of the following steps:

15       a) putting a polynucleotide according to Claim 7 or 8 as probe in contact with a biological sample;

      b) detection and/or measurement of the hybrid formed between said probe and the DNA of the biological sample.

25. Kit for making use of a method according to Claim 24 in a biological sample characterized in that it comprises the following parts:

      a) a polynucleotide according to Claim 7 or 8 used as probe;

25       b) the reagents necessary for using a hybridization reaction.

26. Method according to Claims 20, 22 and 24 for the diagnosis of cellular proliferation.

27. Ligand screening method likely to affect the transcription activity of a gene the promoter of which 30 consists of CCAAT and/or inverted CCAAT boxes (ICB) likely to bind a polypeptide according to Claims 1 to 4.

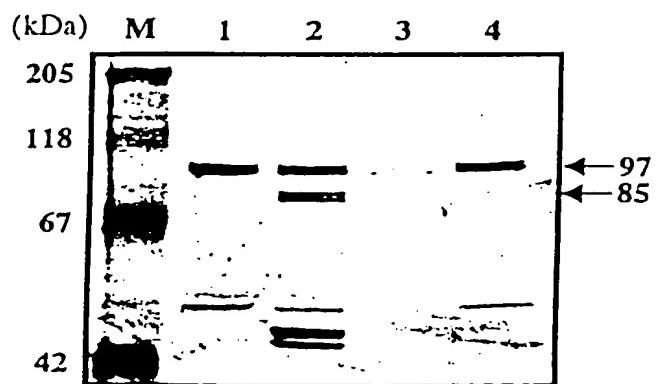


FIG. 1

CABINET REGIMBEAU

**DUPLICATA**

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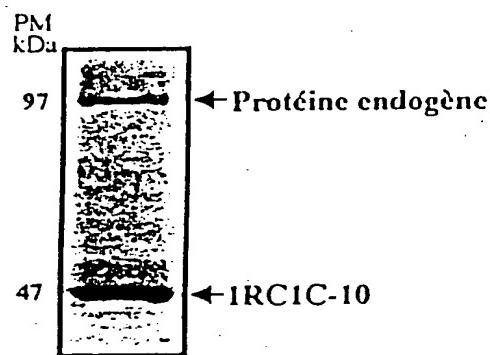


FIG. 2

CABINET REGIMBEAU  
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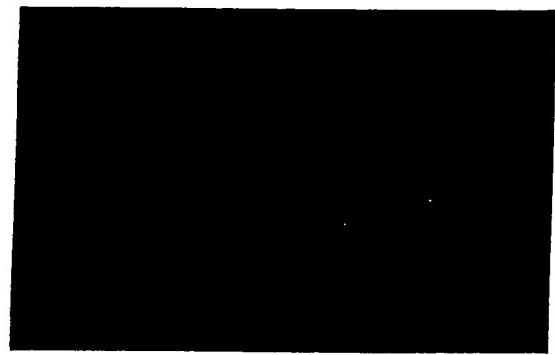


FIG. 3

CABINET REGIMBEAU  
**DUPLICATA**  
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FIG. 4

CABINET REGIMBEAU

**DUPLICATA**

certifié conforme à l'original

|   | 1                            | 2                       | 3                         | 4                        | 5                   | 6                           | 7                      | 8                      |
|---|------------------------------|-------------------------|---------------------------|--------------------------|---------------------|-----------------------------|------------------------|------------------------|
| A |                              |                         |                           |                          |                     |                             |                        |                        |
| B |                              |                         |                           |                          |                     |                             |                        |                        |
| C |                              |                         |                           |                          |                     |                             |                        |                        |
| D |                              |                         |                           |                          |                     |                             |                        |                        |
| E |                              |                         |                           |                          |                     |                             |                        |                        |
| F |                              |                         |                           |                          |                     |                             |                        |                        |
| G |                              |                         |                           |                          |                     |                             |                        |                        |
| H |                              |                         |                           |                          |                     |                             |                        |                        |
| A | adult<br>brain               | amygdala                | caudate<br>nucleus        | cere-<br>bellum          | cerebral<br>cortex  | frontal<br>lobe             | hippo-<br>campus       | medulla<br>oblongata   |
| B | anterior<br>lobe             | putamen                 | substantia<br>nigra       | temporal<br>lobe         | thalamus            | sub-<br>thalamic<br>nucleus | spinal<br>cord         |                        |
| C | heart                        | aorta                   | skeletal<br>muscle        | colon                    | bladder             | uterus                      | prostate               | stomach                |
| D | liver                        | ovary                   | pancreas                  | pituitary<br>gland       | adrenal<br>gland    | thyroid<br>gland            | salivary<br>gland      | mammary<br>gland       |
| E | kidney                       | liver                   | small<br>intestine        | spleen                   | lymph               | peripheral<br>leukocyte     | lymph<br>node          | bone<br>marrow         |
| F | appendix                     | lung                    | trachea                   | placenta                 |                     |                             |                        |                        |
| G | fetal<br>brain               | fetal<br>heart          | fetal<br>kidney           | fetal<br>liver           | fetal<br>spleen     | fetal<br>thymus             | fetal<br>lung          |                        |
| H | yeast<br>total RNA<br>100 ng | yeast<br>tRNA<br>100 ng | E. coli<br>rRNA<br>100 ng | E. coli<br>DNA<br>100 ng | Poly r(A)<br>100 ng | human<br>Cell DNA<br>100 ng | human<br>DNA<br>100 ng | human<br>DNA<br>500 ng |

CABINET REGIMBEAU

**DUPLICATA**

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FIG. 5

|      | 10          | 20          | 30          | 40         | 50          | 60          | 70          | 80          |
|------|-------------|-------------|-------------|------------|-------------|-------------|-------------|-------------|
| 1    | ATGTCGATCC  | ACGTTGGCAC  | CATGGATCGG  | AGCCAGACCC | ACACGGTOGA  | CTCCGCTGTC  | ACGGCTGACCA | ACGTGGACCA  |
| 81   | GCTGACCGG   | AAGATOCACG  | ACCTCTTCCA  | CGTGGACCCA | GGCCCTCCAGA | GGCTGTTCTA  | CAGGGCCAAA  | CACATGGACG  |
| 161  | ACGGCCTAC   | CCTCTTCGAC  | TACGAGATCC  | GCCTGAATCA | CACCATCCAG  | CTCCCTGCTCC | CCCAGAGCCT  | CGTGGCTCCCC |
| 241  | CACACCCACCA | ACGGACGGGA  | CTCCGAACTC  | TCCCACACCG | ACTCCGGCTG  | CTGCCCTGCC  | CAGACTGAGT  | CACACAAGTC  |
| 321  | CTCCACCCAC  | GGTGAOGCCG  | CCGCGGAGAC  | TGACAGCAGG | CCAOCCGATG  | ACGACATGTC  | GGATGAGACG  | GAATTGGGGC  |
| 401  | TGTACAAAGGT | CAATGACTAC  | GTGCACTTC   | GGGACACCAA | CATGGGGCG   | TGGTTTGAGC  | CCGAGCTGGT  | CACGGTGACG  |
| 481  | CGGAAGCCCC  | CCTCCCGGGA  | CGAGCCCTGC  | AGCTCCACGT | CCAGGGCCG   | GTCGGAGGAG  | GACCTCATTT  | ACACAGTGAA  |
| 561  | ATACGACGAC  | TACCCCGAGA  | ACGGGGTGGT  | CCAGATGAAC | TCCAGGGACG  | TCCGAGCCG   | CGCCCCCACC  | ATCATCAAGT  |
| 641  | GGCAAGACCT  | GGAGGTGGCC  | CAGCTGOTCA  | TGCTCAACTA | CAACCCCGAC  | AACCCCAAGC  | ACGGGGCTT   | CTGGTACCGAC |
| 721  | GGGGAGATCT  | CCAGGAGACG  | CGAGACCAAG  | ACGGGGCGG  | AACTCTACCC  | CAACGTTGTC  | CTGGGGGATG  | ATTCTCTGAA  |
| 801  | CGACTGTCGG  | ATCATCTTC   | TGGACGAAGT  | CTTCAAGATT | GAGCGGGCGG  | GTGAAGGGAG  | CCCCATGOTT  | GACAAACCCCA |
| 881  | TGAACAGGAA  | GAGCGGGCGG  | TCTCTGCAAGC | ACTGCAAGGA | CGACGQTQAAC | AGACTCTGCA  | GGGTCTGGCC  | CTCCCACCTG  |
| 961  | TCCGGGGGCC  | CCGAGGACCC  | CGACAACAG   | CTCATGTCGG | ATGACTCGGA  | CATGACCTTC  | CACATCTACT  | CCCTGGACCC  |
| 1041 | GCCCCCTCAGC | AGTGTTCCTA  | GGAGGAGGA   | GTGCTACTGC | CTCGACTCC   | CGAATGATOC  | CAGCGAGCTG  | GTACTGGGGC  |
| 1121 | GAGACGGGCT  | GAGAGAGACG  | AAGAAGAATG  | CGAACATGCC | CTCGGGCCACA | TGCTCCCTAC  | ACGGGGACTG  | GGCCAAGGGC  |
| 1201 | ATGCCCTCTG  | TGGCCCGCAC  | CAAGGAATGT  | ACCATGTC   | CGTCCAACCA  | CTACGGACCC  | ATCCCCGGGA  | TCCCCGTCGG  |
| 1281 | CACCATGTCG  | CGGTTCCGAG  | TCCAGGTCAG  | CGACTCGCGT | CTCCATCGC   | CCCACGTGCG  | TGGCATCCAT  | GGCCCCGACCA |
| 1361 | ACGAOGGGATC | GTACTCCCTA  | GTCCCTCGGG  | GGGGCTATGA | CGATGATOTG  | GACCATGGCA  | ATTTTTTCAC  | ATACACGGGT  |
| 1441 | AGTGCTGGTC  | GACATCTTTC  | CGGCAACAAAG | ACGACCCGGG | AACAGTCTG   | TGATCAGAAA  | CTCACCAACA  | CCAACAGGGC  |
| 1521 | CCTGGCTCTC  | ACTGCTTTG   | CTCCCATCAA  | TGACCAACAA | CGGGGGCAGG  | CCAAGGACTG  | GGGGTCCGGG  | AACCCGGTCA  |
| 1601 | GGCTCTCTGG  | CAATGTCAG   | GTGCGGAAGA  | ATACCAACTA | CGGGGGCGT   | GACGGGACCC  | GCTACCGATCG | CATCTACAA   |
| 1681 | CTTGTGAAT   | ACTGGGGCGA  | GAAGGGGAAG  | TCCGGTTTC  | TCGTGTGCG   | CTACCTTCTG  | CGGAGGGAGC  | ATGATGAGCC  |
| 1761 | TGGCCCTTGG  | ACGAAGGAGG  | CGAAGGACCC  | CATCAAGAAG | CTGGGGCTGA  | CCATGCCACTA | TCCAGAAAGC  | TACCTGGAAG  |
| 1841 | CCCTGCCCAA  | CCGAGACCGA  | QAGAAGGAGA  | ACAGCAAGAG | CGAGGAGGAG  | QAGCAOCAG   | AQGGGGCTT   | COCTCCCCC   |
| 1921 | AGGACGGGCA  | AGGGCAATG   | QAGGCGGAAG  | TGCGCAGGAG | GTGGCCGAG   | CGGGGGGGG   | TCCCCCGCCC  | GGACATCCAA  |
| 2001 | AAAAACCAAG  | GTGGAGCCCT  | ACAOTCTCAC  | GGCCCAQCAQ | AOCACTCTCA  | TCAAGAGAGA  | CAAGACCAAC  | QCCAACTGT   |
| 2081 | GGATGAGGT   | CCTGGCGCTCA | CTCAAGGACG  | GGCCCGCGAG | CGCGAGCCG   | TTCCAACTGT  | TGCTGAGTAA  | AGTGGAGGAG  |
| 2161 | ACOTTCAGT   | GTATCTGCT   | TCAGGAGCTG  | GTGTTCCGGC | CCATCACGAC  | GTGTTCCAG   | CACAACTGT   | QCAAGGACTG  |
| 2241 | CCTGGACAGA  | TCTTTCTGG   | CACAGGTTT   | CAOCTCCCT  | QCCCTCCCGT  | ACGACCTGGG  | CGGCACTAT   | QCCATOCAGG  |
| 2321 | TGAACCAAGC  | TCTGCAAGACC | GTCTCAACC   | AOCTCTTCCC | CGGCTACCGG  | AAATGCCGQT  | GA          | 2382        |
|      | 10          | 20          | 30          | 40         | 50          | 60          | 70          | 80          |

FIG. 6

**CABINET REGIMEAU**  
**DUPLICATA**  
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|     | 10         | 20         | 30         | 40         |            | 50         | 60          | 70          | 80  |
|-----|------------|------------|------------|------------|------------|------------|-------------|-------------|-----|
| 1   | MWIQVRTMDG | ROHTVDSL   | RLTKVEELRR | KIOELPHVEP | GLORLYRCK  | QMEDGHTLFD | YEVRLNDTIQ  | LLVRQSLVLP  | 80  |
| 81  | HSTKERDSEL | SDTDSCCCLG | QSESDKSSTH | GEAAAETDSR | PADEDHWIET | ELGLYKVNEY | VARDTNMCA   | WTEAQVVRVT  | 160 |
| 161 | RKAPSROPC  | SSTSRALEE  | DVIYHVKYDD | YPENGVVQMN | SRDVRARART | IIKWQDLEV  | QVVMLNYPD   | NPKERCFWYD  | 240 |
| 241 | AEISRKRETR | TARELYANVV | LGDD8LNDCR | IIFVDEVFKI | ERPGEGBPMV | DNPMRRKSOP | BCKHCKDDVN  | RLCRVCACEL  | 320 |
| 321 | CGGRQDPDKQ | LWCDECDAF  | HIYCLDPPLS | 8VPSEDEMVC | PECRNDABEV | VLAGERLRES | KKNAKMASAT  | 888QRDMGKG  | 400 |
| 401 | MACVORTKEC | TIVPSNHYGP | IPGIPVGTHW | RFRVQVSESG | VERPHVAGIH | GR8NDG8YEL | VLAOGTYEDDV | D8GMFTTYTG  | 480 |
| 481 | 80GRDL8ONK | RTAEQ8CDQK | LNTNRLAL   | NCFAPINDQE | QAEAKDWRSQ | KPVVVBNVK  | QOKNSKYAPA  | EQMEYDGITYK | 560 |
| 561 | VVKTMPEKOK | 80FLVWRYLL | RRDDDEPGPM | TKEGKDRIKK | LOLTMQTPEG | YLEALANRER | EKENSKREEE  | EQQEEOGFASP | 640 |
| 641 | RTOKOKWKRK | SACCOPEBAG | SPRRTSKTK  | VEPI8LTAQQ | SSLIREDKSN | AKLWNEVLAS | LKDPRPASQSP | FOLFLSKVEE  | 720 |
| 721 | TFQCICCQEL | VFRPITTVCQ | BNVCKDCLDR | SPRAQVFSCP | ACRYDLGRBT | AMQVNQPLQT | VLNQLPPGYQ  | NCR*        | 794 |
|     | 10         | 20         | 30         | 40         | 50         | 60         | 70          | 80          |     |

FIG. 7

CABINET REGIMBEAU

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